THE MOLECULAR ARCHITECTURE *of* PLANT CELL WALLS

Electron micrograph of the outermost lamellae in the Wall of *Valonia ventricosa*. Pd-Au shadowed; magnification 25,000 ×.

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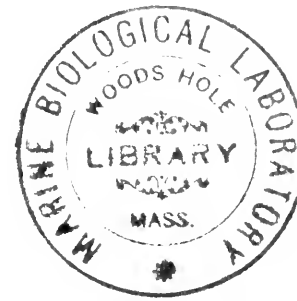
To all the members of my family who, in their various ways, have given me encouragement this book is humbly dedicated



"FOR WHAT we obtain of *Nature*, we must not do it by commanding but by courting of Her. Those that woo Her, may possibly have her for their *Wife*: but She is not so common, as to prostitute her self to the best behaved *Wit*, which only practiseth upon it self, and is not applied to her. I mean, that wherever Men will go beyond Phansie and Imagination, depending upon the Conduct of *Divine Wisdom*, they must Labour, Hope and Persevere. And as the *Means* propounded, are all necessary, so they may, in some measure, prove effectual. How far, I promise not; the way is long and dark; and as Travellers sometimes among Mountains, by gaining the top of one, are so far from their journey's end; that they only come to see that another lies before them: so the way of *Nature*, is so impervious, and, as I may say, down Hill and Up Hill, that how far soever we go, yet the surmounting of one difficulty, is wont still to give us the prospect of another."

NEHEMIAH GREW, M.D., F.R.S.
Anatomy of Plants, 1682.

Preface

THE STRUCTURAL features of cellulose and of the substances associated with it have been the subject of much intensive study for at least thirty years and have given rise to a literature now so enormous that it is difficult even for the expert to keep up with it. From time to time various sections of this study have been reviewed in text-books, but a good deal is still available only in the original papers. In particular no text-book has yet appeared in English confined to those aspects of wall studies of greatest appeal to botanists, and the growing demand for such an account has stimulated me to write the present book.

I have made no serious attempt to cover the ground already so adequately surveyed in a number of texts. The methods and results of physico-chemical investigations of cellulose have already been presented in a series of excellent treatises, and the most recent book by Frey-Wyssling has already laid down the basis of the botanical approach. Nevertheless there remains much that is of importance still not presented, and a good deal of information has already become available even in the short time which has elapsed since Frey-Wyssling's book appeared.

Since the present book is written, however, chiefly for botanists, it has been necessary to present in the first few chapters a brief and, it is feared, wholly inadequate résumé of the more important physical and chemical approaches to cellulose structure. At the same time it is hoped that it may prove of interest also to physical scientists and, though no specific reference is made to any of the obvious technological connections, also to fibre technologists; and for this reason some explanatory account is also given of the anatomy and development of the tissues under review. The rest of the book is concerned with the detailed architecture of cell walls in a wide variety of plants, including growing cells, and an attempt is made to interpret growth processes in terms of the structure thus revealed.

A good deal of the work described in these later chapters has been performed in my laboratory and I have not hesitated to draw on the latest work of my colleagues to whom I owe a very great debt of gratitude. I would particularly mention Dr. M. Middlebrook and Dr. M. F. E. Nicolai, who are still with me, and Dr. K. Singh, now at Dehra Dun, India, and Dr. A. B. Wardrop, now in the Forest Products

Division, C.S.I.R.O., Melbourne, Australia. In particular I would say that I have not hesitated to express opinions on controversial points and I hope they will forgive me if I have taken undue liberties. The inclusion of a good deal of hitherto unpublished material has possibly led me into statements which may eventually need to be modified: but again I have preferred to liven the book at the risk of some later corrections rather than adhere rigidly to fully established interpretations.

I am deeply indebted to Mrs. F. R. Langstadt for the very able way in which she has converted my almost illegible pages into typescript, and to Mr. B. Clarke for the preparation of many of the prints with which the book is illustrated and in particular for the colour plate (Plate III). To Dr. M. F. E. Nicolai, Dr. L. C. Spark and Miss L. I. Scott I am grateful for assistance in proof-reading; any errors which remain are my own responsibility.

It has been my privilege during my research life to be in close association with some of the leading authorities in various fields of science. To all of them, and particularly to Professor W. T. Astbury and, of late years, to Professor I. Manton, I owe a debt I can never repay. It is my sorrow and misfortune that I can no longer convey an expression of my gratitude to the one to whom I owe the most—the late Professor J. H. Priestley.

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CHAPTER I

Introduction and Historical Background

THE THEME of this book centres round the extraordinary advances which have been made, during the past twenty-five years, in the understanding of the molecular structure of the solid envelope which surrounds every plant cell. It is fitting therefore, that we should consider in the first place some of the multitudinous reasons for the current preoccupation with such a topic. Reasons in plenty are not far to seek—they must in fact be obvious after a little thought even amongst those of us unacquainted with plant science. From time immemorial man has made use of plants, not only for food—for that would not help us here since the bulk of the material we shall deal with is not digestible in man's alimentary tract—but also in many other ways. From the Garden of Eden downwards, use has been made of plant products to cover human nakedness, a use widened enormously in scope by the discovery of weaving since fibres of all kinds could then be manufactured into sheets of cloth. Nowadays we are familiar with the weaving of flax fibres into linen, with the weaving of hemp into ropes and jute into bags and with the multitudinous uses of cotton fibres. All of these processes exploit the very fibres which we shall be investigating here. More than this, however, from times well before recorded history man has made use of another plant product—timber—for the building of houses, for furniture, and even for weapons of offence and defence. He has become acquainted with the great strength and durability of such plant products and has made use of their peculiarities, of the lightness and resilience of wood for instance. There can be no doubt but that the peculiar combination of physical properties in these materials—and this short list does not by any means cover all the queer mixture of properties—is due in no small degree to the molecular structure of plant products and, in particular, to the structure of the cellulose so ubiquitous in all of them. As in many other branches of human endeavour, the uses of these materials, and knowledge and exploitation of their peculiarities, came long before any attempt could be made to explain them. Nevertheless explanation is surely needed, and all the more surely in this modern age when so much of our economy depends on the faultless

processing of huge quantities. Without such explanation it becomes impossible to control adequately the varied, and nowadays very complicated, treatments which the raw material receives; and, in particular, if anything goes wrong it is not otherwise easy to put it right again without serious loss. Finally, it is impossible to devise new uses, or to explore the old ones thoroughly, without a good deal of ordered and accurate information concerning the most intimate details of the materials concerned. Realization of these matters has led to the founding throughout the world, in the growing areas as well as in the processing, of scientific laboratories devoted to the problems involved.

This is by no means all, however, and this fails by a long way to exhaust the reasons for study of this particular field; it is certainly not the major reason for writing this book nor does it express in any large measure the fascination of the subject for the author, for any of those whose help it will be an honour to acknowledge, or for any of the long sequence of scholars—for scholars they are even though also scientists! —whose names will grace these pages and in whose footsteps the author and his associates now humbly tread. Apart altogether from its immense impact on the welfare of human beings, a knowledge of how things grow, whether animal or plant, can hardly fail to be of interest to all of us, and this means in the long run a knowledge of the reactions of the protoplasm—the stuff of which all living things are composed and by whose activity they develop. There are naturally many avenues along which such a study can properly be approached, and are being approached; but none of them can be more fundamental than the approach through structure. Until the structure of the living material is fully understood there can be no real appreciation of the course of growth. From this point of view, and since in very general terms protoplasm is very much the same in whatever body it is organized, it is immaterial whether we concern ourselves with plants or with animals, and in some respects plants offer more favourable material for exploratory purposes. Just as animal bodies produce structural proteins such as hair, whose study at the hands largely of Professor W. T. Astbury has led to such sweeping developments in the field of protein physics and chemistry, so in plants we find structural polysaccharides. These are, chemically speaking, a far cry from the proteins, and therefore several steps removed from the molecular species which undoubtedly confer upon protoplasm its particular and still cryptic features. Nevertheless their production in intimate contact with the protoplasm makes it very probable that, as an end product in carbohydrate metabolism, they have a good deal to tell us concerning protoplasmic structure and

activity if only we can read them aright. Further, and of more immediate importance, forming as they do an outer envelope surrounding each little unit of protoplasm in the plant body and remaining so from the beginning during the whole of its life, they can hardly fail to be vitally concerned in the increase in bulk of cells and tissues and to carry with them a record of cellular activity. It is from these points of view, and these alone, that this book has been written.

The astounding progress during recent years in the whole field of structure in biological materials, using the methods which modern physics has placed in our hands, is perhaps sometimes apt to blind us to the very solid foundation which our distinguished predecessors have laid with their completely inadequate tools, and upon whose firmness of construction our ivory towers depend. It is therefore a salutary lesson to go down and consider, even if very briefly, the stones—and the rubbish—which lie in the basement. The historical period concerned can be divided roughly into four sub-periods—the period before the discovery of the microscope, of which we shall have nothing to say, the period from the first use of the microscope in about 1666, to the application of the polarizing microscope in about 1830, the period from this time until the year when the method of X-ray analysis began to develop, and the modern period since that time.

The beginnings of cell wall studies

It was naturally only after the improvement by *Robert Hooke* of the microscope recently devised by the *Janssen* brothers, to give reasonable magnification with tolerable definition, that anything useful could be written about our subject. Although *Henshaw* is said to have discovered the vessels in the wood of walnut trees as early as 1661, the study properly begins with the publication by *Hooke* of his *Micrographia* (1667) and the drawing which he there published, and as so often figured in later treatises of elementary botany, of cell structure in cork. *Hooke*, however, was not in any sense a botanist and contented himself with the description of the honeycomb structure he perceived and with fanciful comparisons with bone-lace. The few years which followed, however, saw rapid advances though almost solely at the hands of two investigators, *Malpighi* in Italy and *Grew* in England. These two together laid the foundation of all that was known concerning cellular structure for the next hundred years. *Grew* in particular, though his presentation lacked the tasteful elegance of *Malpighi*, produced a mass of minute detail on the anatomical features of plants, and we shall confine our attention to him for that reason. Among the many cell

types he saw and figured for the first time (he was the first to use the term parenchyma for instance) he gave early attention to the walls of vessels. On mechanical treatment of these he found them to unwind like flat ribbons and of these ribbons he says (1682) (Plate I):

> . . . the Vessles, oftentimes, unroave in the form of a Plate. As if we should imagine a piece of fine narrow Ribband, to be woun'd spirally, and Edg to Edg, round about a Stick; and so, the Stick being drawn out, the Ribband to be left in the Figure of a Tube, answerable to an Aer-Vessel. For that which, upon the unroaving of the Vessel, seems to be a Plate, or one single Piece, is, as it were, a Natural Ribband, consisting of several pieces, that is, a certain number of Threds or Round Fibres, standing parallel, as the Threds do in an Artificial Ribband. And as in a Ribband, so here, the Fibres which make the Warp, and which are Spirally continu'd; although they run parallel, yet are not coalescent; but contained together, by other Transverse Fibres in the place of a Woof.

He became convinced that all other cell types follow this structure, and concluded in general that the threads in parenchyma cell walls lie horizontally while those of fibres lie vertically. Let us notice particularly the fineness to which he considered these threads could be split.

> So in the Pith of a Bulrush of the Common Thistle, and some other Plants; not only the threds of which the Bladders; but also the single Fibres, of which the Threds are composed; may sometimes with the help of a good Glass, be distinctly seen. Yet one of these Fibres, may reasonably be computed to be a Thousand times smaller than an Horse-Hair.

This latter estimate can hardly be accepted since the fibres would then have been of the order of 0·1 or 0·2μ in diameter. It does seem reasonable to assume, however, that *Grew* had seen threads grading in fineness down to the limits observable. Some two hundred years later *Sachs*, the foremost plant physiologist of his time, was to ridicule these suggestions made by *Grew*; yet it is very instructive to examine his figures, of which one is presented in Plate I, in the light of modern observations with an electron microscope (Frontispiece). It is always easy, of course, to read modern ideas into older and vaguer writings, but here *Grew* expresses himself so unequivocally that one can hardly avoid the conclusion that this interpretation, in terms of what we would now call fibrils, was inspired vision. We are to see the idea of fibrillar structure turning up again and again in the years that followed, both in the wall and in the protoplasm, and equally often being ridiculed. *Grew* was undoubtedly an acute observer; among other things, he realized that the walls of parenchyma cells were complete, without perforations, a point

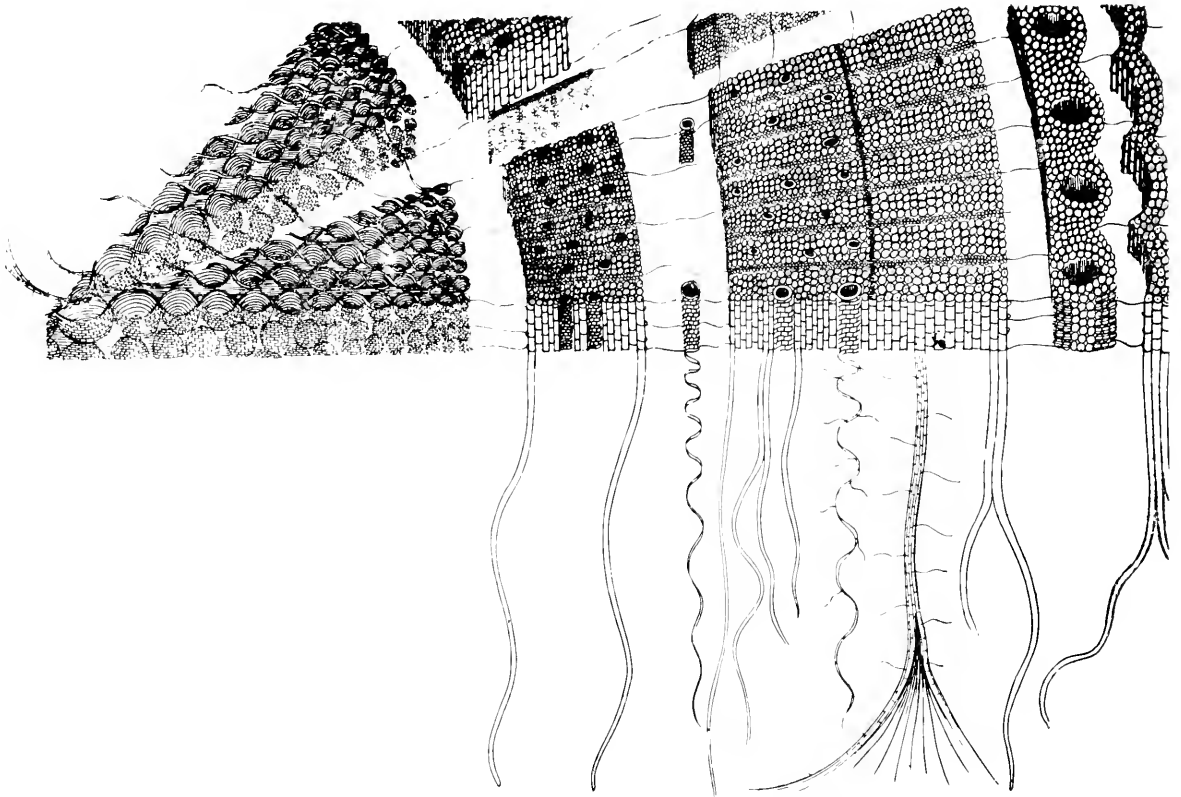

FIG. 1. The structure of the stem of an Angiosperm as figured by *Grew* (1682).
Note the fine fibrils which he considered as forming the structural framework of the cell walls.

[*Facing p.* 4

PLATE I

of the first importance in cellular organization which was not universally accepted for more than a hundred years after his publications.

Advances following improved techniques

The eighteenth century was generally one of stagnation in plant morphology, and even of retrogression as far as wall studies were concerned. Attention was centred around physiological problems, and such details of anatomy as were needed were all too frequently taken bodily from *Grew*'s work. We may perhaps note what seems nowadays a peculiar notion put forward by *Wolff* (1759) that the young parts of plants consist of a transparent gelatinous substance, in which drops of sap are secreted which grow into cells. The lamina separating two cells he therefore regarded as single—an error which was to take many years to eradicate. While this was undoubtedly an honest attempt to make something out of the much more difficult softer plant tissues and was occasionally revived even at a much later date, we cannot but regard it, in the light of the elegance of *Malpighi* and the scrupulous care of *Grew* nearly a hundred years earlier, as misinterpretation of faulty observations. It formed the first denial of the fibrillar hypothesis.

Further development had to await improvements in microscope design and technique and these were not forthcoming until the first quarter of the nineteenth century. During the period from 1812 to 1828 *Selligue* and *Amici* produced achromatic and aplanatic objectives with three double lenses, and with these new instruments progress became very rapid.

Moldenhawer (the younger) used maceration techniques, and saw for the first time whole cells separated from their neighbours. This brought him immediately into conflict with *Mirbel* who (1801) had supported the earlier notions of *Wolff*. Again with *Moldenhawer* we revert in some degree to the "fibrillar hypothesis", since he considered the cells to be held together in tissues by a matrix of finely woven fibres, which indeed he claimed actually to have seen. This reversion culminated with *Meyen* who visualized cell construction very much in the way *Grew* had done. We may perhaps note in passing that *Kieser* (1815) examining the problems of cell shape which have been a subject of investigation even in the most recent times, pointed out (almost correctly) that all cells were fundamentally rhombic dodecahedra.

Undoubtedly the greatest figure of this period, however, was *von Mohl*. He gave what amounts to the modern view both on the primary and the secondary walls of cells (*see* Chapter II) though he misunderstood the bordered pit, and one feels in his work, perhaps for the first

time, the security which comes from scrupulous care in observation and a revulsion against unwarranted generalizations and abstractions. He was the first to use the polarizing microscope to any purpose in this study and, though he cannot be said in any way to have anticipated *Nägeli*, his interpretation of striations (fine markings seen on some walls) in terms of lines of cleavage in crystals reveals the lines upon which he was thinking. He considered the secondary wall to be deposited as a series of lamellae laid one upon the other by a process which he therefore called *apposition*, a notion which fitted in so admirably with the conclusions of *Payen* (1844) that young cell walls consisted of rather pure cellulose, incrusting substance being added later, that together they were able to contest successfully the curious notion of *Mulder* and *Harting* that the innermost layer of a cell wall was the oldest.

By the year 1850 *von Mohl* had come to regard the intercellular substance (the modern middle lamella) as only a cementing material, and other writers (*e.g. Unger*) agreed. Some, however, including *Wigand* and even *Sanio*, regarded it as the primary wall modified chemically, and thus set in motion the confusion of terms which has lasted until well into the twentieth century.

Among many other distinctions of *von Mohl*, we may perhaps note only that he gave the name of protoplasm (1846), first applied by *Purkinje* (1840) to the formative substance of animal eggs, to the living substance inside the cell.

The general impression at this time was therefore of growing cells with thin expanding cell walls of cellulose upon which lamellae were plastered secondarily by apposition. The cells themselves were probably cemented together with a formless cement, but in none of these structures was any attempt made at an understanding of submicroscopic features or therefore at an impression of the processes of growth which such knowledge alone can give. This was, however, soon to be remedied in the capable hands of *Nägeli*.

The modern era

Although *Nägeli* was a contemporary of *von Mohl*, the outstanding success of his application of physical principles to wall problems must single him out as the first of the modern workers. The publication of his *Stärkekörner* in 1858 marked the beginning of a new era in wall studies—and in the field of colloids generally—and of a tradition in structural investigation at Zurich, where he worked, kept up so ably in more recent times by *Frey-Wyssling*. For the first time, *Nägeli*

demonstrated what could be done with the polarizing microscope once the underlying physical principles were fully appreciated. Careful observations with this instrument convinced him that the building material of starch grains, and subsequently of cell walls, was truly crystalline; and later attempts to throw doubt on his interpretations at the hands of *Hofmeister* and even of *Strasburger* were foredoomed to failure. Following his studies of swelling reactions in starch grains, *Nägeli* concluded that they were composed of elongated particles lying radially in the grain and separated by films of water. He deduced that smaller particles should have thicker water films, and the familiar lamellation was (again correctly) interpreted as due to alternation of higher and lower water content. These elongated particles, which *Nägeli* later (1877) called *micelles*, were supposed to be stabilized in these aggregates by the opposing attractions of the grains for each other and for water. His greatest contribution at this time was, however, the idea that starch grains increase in size by the insertion and growth of new micelles between the old, and he soon extended this suggestion to cover cell walls also. Such a process, which he called *Intussusception*, fitted much more easily the conceptions of wall growth at that time than did the *apposition* of *von Mohl*, and received general acclaim. As we shall see later, it is amazing how near *Nägeli* came to a description of wall organization as we know it today.

Not, however, that these ideas were accepted without some scepticism. *Strasburger's* denial of the existence of micelles and of any crystallinity, coupled with his insistence on apposition, had the support of *Noll*, *Klebs*, *Zimmerman* and *Askenasy*. On the other hand, a number of investigators, notably *Haberlandt*, *Zacharias*, *Krabbe* and *Cramer* produced rather convincing evidence in favour of the ideas of *Nägeli*. Finally, *Pfeffer* in 1892 suggested that there might be no universally acceptable mode of wall growth, and it goes without saying that later work showed this to be the solution leading to the modern views which will be discussed later in this book (p. 11 *et seq.*).

During this time, parallel investigations had been going on in the chemistry of the wall. As early as 1825 "cellulose" was known to be a mixture of the substances *cellulose* and *pectose*. The first use of cuprammonium to remove the cellulose was made by *Frémy* (1859) and the subsequent staining and solubility tests soon led to the discovery that the bulk of the pectic substances was located in the middle lamella (*Kabsch*, *Vogl*, 1863) though it must be remembered that at this time it was not clear whether the middle lamella was a real cementing interstitial layer or whether it consisted merely of the two primary walls. *Sanio* in

1873 supported this latter view. The real study of pectic compounds begins, however, with *Mangin* (1892, 1893) who laid the foundation of our present ideas. He showed that there are two series of compounds —acid and neutral—each with several members differing in solubility, and succeeded in demonstrating that all these differed considerably from cellulose in the chemical sense. He showed further that hardly any plant tissue is free of pectin, which normally is associated intimately with the coexistent cellulose but only mechanically and not chemically. He confirmed the earlier view of *Payen* that the middle lamella contains calcium pectate and opposed successfully the ideas of *Strasburger* by claiming that this is a true intermediate layer. Through the work of *Mangin*, and of many others both botanists and chemists, the next twenty years produced much valuable data on the types and distribution of these compounds, but these later developments, as well as the modern treatment of the micellar theory, are best summarized in the body of the book.

This remarkably rapid development in the field of carbohydrate biochemistry and biophysics was naturally paralleled by a similar expansion in our knowledge of protoplasm. Up to about 1864 this remarkable substance had been conceived as a viscid, slimy liquid containing granules, though *Brucke* had concluded that such an appearance must cover some kind of organization. This fluid theory, with modifications, was in fact held by *de Bary*, *Schwendener* and even *Nägeli* up to about 1877, when some botanists (*e.g. Volten*, 1873–6) and zoologists (notably *Schulze* (1871) and later *Flemming* (1882)) were beginning to accept the evidence for the alternative fibrillar structure of protoplasm then coming into favour. Here we meet again the constantly recurring insistence on the fundamentally fibrillar organization of biological substances. An attempt to harmonize the Granular and the Fibrillar Hypotheses, largely at the hands of *Frommann* (1867), led to the conception of protoplasm as fundamentally a network in which the nodal points in the net could give the erroneous impression of granules. This was the so-called Reticular Hypothesis, modified later by *Bütschli*, who interpreted the reticular appearance as the optical expression of a foam structure and proposed the Honeycomb Theory associated with his name. At this time the Reticular Hypothesis in particular received very strong support, though *Pfeffer* in 1890 sounded a warning note in denying that protoplasm could be such a firm, continuous and permanently rigid structure as it was apparently coming to be. Again, *Schwartz* (1887) pointed out that a reticulate appearance, identical with that said to be visible in protoplasm, could be induced in the white of

an egg, and attempted to revive the older fluid theory by ascribing the reticular appearance to precipitations at certain places and under certain conditions. If this can be taken to imply a submicroscopic fibrillar organization which "condenses" into a grosser structure during coagulation, then this is a close approach to the modern view. Towards the close of the nineteenth century this kind of treatment was much in favour though occasional workers such as *Altmann* remained to the last firm upholders of the older granular conception, an idea which was in fact still upheld by *Heilbrunn* in 1926. The arguments against such a proposition were always that the protoplasm would then lack the structural organization which was realized, on physiological grounds, to be essential, just as the argument against the reticular (or alveolar hypothesis, depending on whether one thought the firmer or the less firm phase to be the important one) were aimed at its improbable rigidity. Both sides undoubtedly went too far; protoplasm certainly cannot conceivably be regarded as rigid in any sense, nor can its behaviour be harmonized with that of normal non-Newtonian liquids.

Perhaps at this time, when so little was known of the behaviour of non-Newtonian liquids, the fluid nature of protoplasm was overemphasized. It had already been found by *Pfeffer* (1890–1) that living protoplasm has tensile strength, a property which can hardly be reconciled with normal fluidity; and the very varied results of viscosity measurements published during the present century are clearly in better harmony with the fibrillar or reticular hypothesis than with any postulating a fundamentally fluid nature. Once it was accepted that the physiologically important substances in protoplasm are the proteins (*Leathes*, 1925, *Pauli*, 1922 and many others),* then analogies with substances like gelatine could be drawn and the situation became a little clearer. The way was at last open for an attack on the molecular structure, not of the protoplasm itself, it is true, but of the proteins upon which the variability of protoplasmic behaviour so clearly depends. It is not possible here to detail the remarkable advances in recent years on protein structure, but one or two points should properly be made since we shall need them at various times in the following pages.

The first successful interpretations of the X-ray diagrams of proteins were made by *Astbury* working with the keratin of wool. He showed for the first time that proteins could be considered as long molecular chains of amino-acid residues, folded in some particular configuration. With

* We now suspect that the proteins are not distributed uniformly throughout the protoplasm, and it is still possible that some regions in protoplasm must still be regarded as permanently fluid.

minor modifications, this conception has proved of outstanding success in all of the protein types which *Astbury* and many other workers have investigated since those early days. Naturally, first attention was paid to the structural proteins like keratin which lend themselves more readily to investigation by X-ray methods. Extension to the so-called globular proteins, which resemble much more closely protoplasm itself, led at first to some confusion, since in these proteins the molecules are, as the name implies, globular and not fibrillar, in shape. It seems now, however, widely agreed that these globular bodies consist nevertheless of protein chains, but closely folded in some specific way. As for protoplasm itself, it clearly exhibits properties such as streaming, which would harmonize better with the properties of a globular protein, and others such as tensile strength which would seem in better agreement with the properties of the fibrillar proteins. As *Frey-Wyssling* has pointed out, protoplasm itself may lie half-way between these two extremes and may therefore consist of a loose network of folded polypeptide chains, in which the features of the whole structure depend therefore as much on the state of folding as upon the constitution of the individual proteins.

With this vague conception we may well leave the history of these substances and discuss any further points more fully later as and when they are required.

CHAPTER II

The Form of the Plant Cell

BEFORE PROCEEDING to a discussion of the modern developments in the molecular architecture of the walls of plant cells it will be as well to pass briefly in review the range of structures, in a microscopic sense, with which we shall be concerned. This is all the more desirable, even for those who have already some considerable knowledge of plant anatomy, since the form in which the material is presented in nature is the only form available for study; so that, unlike the state of affairs in corresponding investigations with matter not associated with living things, it is impossible to change to any extent the form of the experimental material. This imposes strict limits upon the kind of things which can be done, particularly in view of the inherent microscopical complexity of even the smallest piece of a tissue which can conveniently be handled.

All plants, like all animals, originate from individual drops of protoplasm which are commonly spherical, or nearly spherical, in shape and are usually such as can comfortably be seen only under a microscope. Such a shape undoubtedly conforms to the liquid, or semi-liquid, consistency of the protoplasm, at least during some stage of its development. In plants, however, unlike the animals, this drop of protoplasm comes, sooner or later, to be covered by a membrane, thin and delicate yet undoubtedly solid, which therefore limits any further rapid changes in shape; and so long therefore as the cell, as we may now call it, remains free from its neighbours and removed from any other obstacle to development, it will remain spherical, or approximately so, provided that the membrane—*the primary wall*—is such that it will expand equally in all directions in response to the same mechanical disturbances. In fact, many cells which do remain free in this way, do retain their almost spherical shape—some unicellular algae, spores of fungi, mosses, ferns, etc.—although in some cases—the filamentous algae for instance —they rapidly become cylindrical in spite of the absence of neighbouring lateral cells.

Such instances as these latter naturally imply that the surrounding solid envelope is not isotropic and this we shall have to examine in some

considerable detail later on as one example of the control of cell shape by wall architecture. When, however, the original single cell develops, by continued division, into a mass of cells which adhere—that is immediately a tissue is formed—then it is understandable that the original spherical shape is lost. The shape which will then be taken up in a homogeneous tissue can be determined in any of three ways. If the tissue is truly homogeneous, *i.e.* if the cells are all of the same shape and size, then the problem resolves itself into the mathematical one of deciding which polyhedra are capable of filling space completely when placed regularly side by side, followed by the subsequent determination of the most probable of the forms which may thus be revealed. Secondly, argument can be made by analogy from inanimate bodies which appear to be developed under the same geometrical conditions, *e.g.* from the froth on soap solutions or even on beer. Here the bubbles which constitute the froth would be truly spherical, if free, and depart from this ideal shape only on account of the presence of neighbouring bubbles. Equally, the compression of closely packed spheres of plasticine or lead shot—even of swelling pea seeds—would yield polyhedra of the type required; finally, and perhaps most unequivocally, it is possible, under certain circumstances, to separate the cells in a homogeneous tissue and observe them from various directions under a microscope. This last type of observation, which might perhaps be expected to give the readiest answer, is in point of fact by no means so easy as it appears. All three methods have, however, been used and, in spite of a good deal of controversy in the past, there is at the moment a mutual agreement most unusual in the biological field. It seems now to be generally agreed that the shapes of all cells are based on the orthic tetrakaidecahedron or cubo-octahedron almost in the form, therefore, suggested by Kieser more than 100 years ago. This was originally suggested by Lord Kelvin as very nearly the form soap bubbles assume when filling space completely and is shown in Fig. 1. There is, however, one slight modification to be made in this conventional figure in that the eight hexagonal faces are not plane but are slightly curved producing the so-called "body of Thomson". This has also been reported to be the case in plant cells (1).

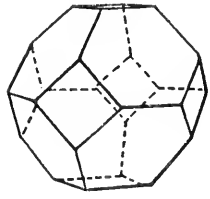

FIG. 1. Diagrammatic representation of the ideal shape of meristematic cells. The polyhedron has eight hexagonal and six tetragonal faces.

It is not, of course, to be assumed for a moment that all the cells in a naturally occurring tissue will adopt this ideal shape. The mere fact of

division, implying that the cells are in fact not all of the same size, and in any case periodically reducing the number of faces by the insertion of a new face diametrically across such a 14-sided polyhedron, will assure some divergence in shape; but nevertheless it must be borne in mind that cells do approximate to this shape or to some derivative of it. Obviously any derivative of the orthic tetrakaidecahedron produced by parallel displacements will equally well fill space and, in particular, the polyhedron can be elongated in any direction without losing its

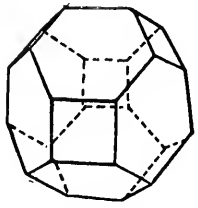

FIG. 2 (*a*)

FIG. 2(*b*)

FIG. 2. Diagrammatic representation of a cell of the apical meristem and a cell produced from it by elongation of four hexagonal and two tetragonal faces in the same zone. Note that both cells have an axis of twofold symmetry parallel to the length of the page. For convenience the isodiametric and the elongated cell are drawn in the same orientation; it does not necessarily follow that only cells oriented as in Fig. 2(*a*) could give cells as in Fig. 2(*b*), since elongated cells will take up the form illustrated in the latter figure, on account of the presence of neighbouring cells, irrespective of the orientation of the parent cell.

space-filling power. This is what in fact usually happens in the development of fibrous cells and one possibility, founded on the careful observation of Lewis (2), is shown in Fig. 2.

In the growing apices of roots and shoots, where cell division is occurring rapidly, then, the cells will approximate to more or less regular 14-sided polyhedra. In the development of stem and root, however, some of the facets of some of these polyhedra begin to elongate. It is not proposed here to examine at all the factors which cause such cells to elongate rather than to swell up uniformly, but merely for the moment to accept the fact of elongation itself. This unidirectional expansion of six faces of the polyhedron (Fig. (2*b*)), without change in their general form, implies that four of the longitudinal faces of an elongated cell are effectively hexagonal, and two tetragonal, provided that the cell tissue still fills space completely. The cell as a

whole, therefore, has only twofold symmetry about its longitudinal axis (*i.e.* the cell will be coincident with itself only twice per revolution about the longitudinal axis) but even this implies that opposite walls should be similar in all respects though adjacent walls need not be. The argument here would be that since the opposite walls presumably began alike and have gone through the same deformations then the final structures must be alike. It is, of course, a natural consequence of growth that as the cell elongates it may develop new contacts and therefore new facets, and some of these can be seen in the beautiful plaster casts of elongated cells prepared by Lewis (2); and in any case as cells vacuolate they develop between themselves intercellular spaces—usually rather small in a tissue of elongated cells—but neither of these minor changes should be allowed to mask the fundamental underlying symmetry. In many elongated cells, in fact, the cells are so long that if their tips be ignored then the longitudinal axis is a sixfold axis, since the shape of the faces is masked, or even more when intercellular spaces develop so that the cells become almost cylindrical; and we should expect in these cases that the longitudinal walls would be constructed on some uniform plan. This is actually realized in some wood fibres and in phloem fibres. In other cases lateral extension of an opposite pair of walls, or some other effects, accentuate the twofold symmetry and we might expect in this case adjacent longitudinal walls to differ in structure even though opposite walls are identical. Both these conceptions are realized, the latter more particularly in the wood tracheids of conifer stems.

During this extension, the cell remains clothed in the original thin membrane which must therefore in some way be able to accommodate itself to change in dimension as the protoplasm increases in volume. The relationship between this primary wall and the protoplasm is, in fact, highly complex and we shall have occasion to examine it in much more detail later on. At the moment we may merely note that up to this time the wall and the protoplasm adhere together rather strongly. This is evidenced by the fact that if young growing cells are placed in a strong sugar solution then, as the solution draws water from the cell by osmosis, the whole cell often crumples without any cleavage between wall and protoplasm; whereas in an adult cell a similar procedure causes the protoplasm and the wall to separate in the phenomenon known as *plasmolysis*. There is much more evidence than this, consideration of which may be left until later when the organization of these walls has been considered.

During the elongation period the cell wall has not, as far as one can tell under the microscope, become any thinner. At this stage the

membrane separating two protoplasts consists of three layers—the *primary wall* of each cell together with the cementing material, *middle lamella*, between. These three layers together are so thin that measurement becomes very inaccurate and it is therefore somewhat difficult to tell precisely what is happening. Chemical analysis, which

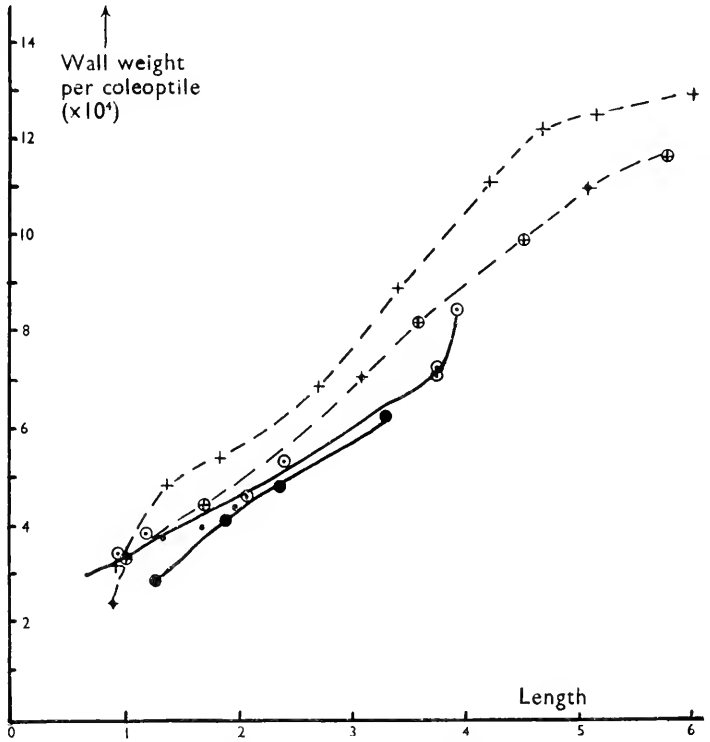

FIG. 3(*a*). The relation between wall weight per coleoptile (gm. $\times 10^4$) and coleoptile length (cm.) during elongation by vacuolation. Weight in gm. ($\times 10^4$) (Preston and Clarke).

—⊙— crop in light at 23°C. + crop in dark at 10°C.
—●— crop in light at 20°C. ✦ crop in dark at 23°C.
—●— crop in light at 30°C. ⊕ crop in dark at 30°C.

Note that the wall weight increases over the whole growth period. The pronounced upward curve after four days in the crop grown in light at 23°C. is associated with a cessation of growth in length at the end of four days under these conditions.

would give the answer, is commonly rather difficult to carry out on account of the difficulty in culturing a tissue of exactly equivalent cells going through the same stages of development simultaneously, but this can partially be achieved with coleoptiles. These are tubular organs

which form the outer covering of the plumule of germinating cereal grains. After the coleoptiles have reached a length of 1 cm., further growth is exclusively by vacuolation, *i.e.* increase in water content per cell, leading to an increase in vacuole size and increase in cell length. Under carefully controlled conditions, a whole batch of coleoptiles can be persuaded to grow at the same rate. This provides very

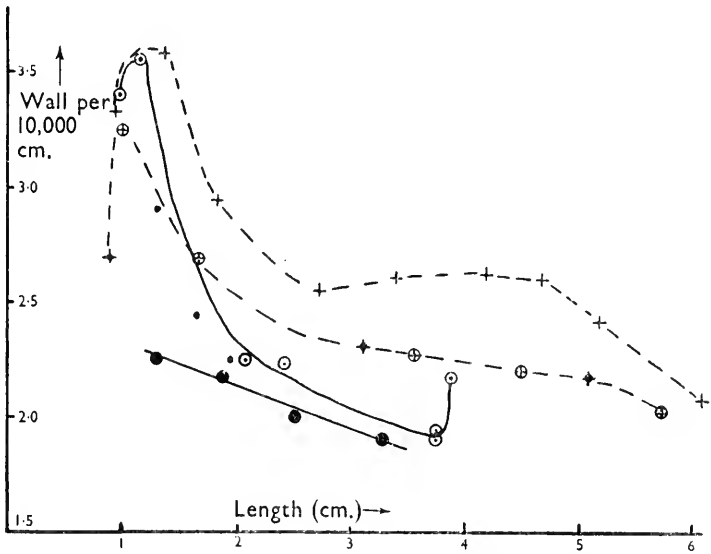

FIG. 3(*b*). The relation between wall weight (gm.) per unit coleoptile length and coleoptile length (Preston and Clarke). Conventions as in Fig. 3(*a*). After a length of 1 cm. has been reached, the wall weight decreases continually apart from a halt lasting from a length of 3 cm. to a length of almost 5 cm. in crops grown in the dark at 10° C. Note again that the upward turn at the end of the light 23° C. series is associated with a cessation of growth in length.

convenient material for a study of cell elongation, since samples can be harvested at intervals for analysis. The results of one such analysis are presented in Fig. 3. It will be clear that the wall weight per coleoptile, and therefore per cell, increases regularly throughout the whole growing phase (Fig. 3(*a*)) so that there can be no doubt but that new wall material is laid down during growth. There is, on the other hand, the further peculiar fact that the wall weight per unit length of coleoptile, and therefore approximately per unit cell length, decreases continually over the growth phase (Fig. (3*b*)). Such wall deposition as occurs during growth, therefore, fails to keep pace with dimension changes. This is a point which must be considered later on. At the moment it is merely to be noted that, in the particular case of oat coleoptiles, while the

wall apparently does not become thinner during growth, its solid substance does diminish per unit area even though the cell continues to make new wall substance. It is always dangerous to argue from isolated cases such as this, but the morphological similarities between growing plant tissues make it seem possible that this may be a general phenomenon. The new wall material which has been deposited must have been laid down by one of two processes—either by plastering new layers from within (the so-called *apposition* of von Mohl) or the insertion, within the existing wall, of new particles of wall substance (the so-called *intussusception* of Nägeli). As the cell ceases to grow, however, the wall certainly begins to thicken by the deposition of new layers from within, and at about this time the cell begins to show the phenomenon of plasmolysis. Now, therefore, that an interface appears to have developed between the cell wall and the protoplasm the new wall layers may be thought of as deposited at a protoplasmic surface. The new layers differ from the primary wall in several respects. They can often be distinguished from it under the microscope in untreated transverse sections and must therefore have different refractive indices, which alone would indicate a difference in the submicroscopic structure, and they often show different staining reactions. By now the cell, whether elongated or not, contains a large vacuole which fills it almost completely, and at this stage the cell wall is thought of as being characterized by the presence of a thin lining of protoplasm on its inner face, separating it from the vacuole. The secondary wall now proceeds to develop to an extent which varies according to the type of cell considered. In the isodiametric cells of parenchyma, the secondary wall commonly remains thin even though the protoplasm remains alive for some considerable period and retains its metabolic activity as indicated by the storage within it of starch. In other cells, secondary wall production proceeds until almost the whole cell volume is occupied by the wall; this occurs in many phloem fibres for instance, where the cell therefore sometimes looks like a solid rod with a narrow thread-like cavity running down the centre. In the majority of elongated cells —wood fibres and tracheids, collenchyma cells, many phloem fibres— and in some which are not so elongated—vessels in dicotyledons, vegetative cells in algae, etc.—the wall becomes appreciably thick but the protoplasm either degenerates or ceases to deposit cellulose before the cell becomes filled. In the elongated cells of the higher plants— —*e.g.* wood fibres and tracheids—the protoplasm actually dies and the cell contents disappear, leaving a hollow thread consisting of the thickened wall envelope surrounding the *lumen*. It is with cells of this

kind that the investigations described in the following pages are largely concerned.

The secondary wall which thus surrounds the lumen in this condition is usually rather complicated in structure. It can, however, vary from the apparently simple and homogeneous (as in some vessels) to a complex of several concentric lamellae alternating either in chemical make-up or in physical constitution or both. Normally these layers are distinguishable under the ordinary microscope even without pre-treatment, but in many cases special procedures have to be adopted, such as staining and the like, in order to make them out at all. Invariably the visible differences arise through a complex of many underlying chemical and physical factors and it will be a large part of the endeavours here to sort out the various factors which impart these differences to the layers and therefore to the cells as a whole. In addition to this complexity in the fundamental building material of the wall layers there are other disturbances of structure which, although perhaps not of such immediate importance to an understanding of the fundamentals of wall structure, must nevertheless be taken into account, particularly when attempting to define the properties of single walls from those of a whole tissue, however homogeneous. In the majority of cell types the secondary wall is not uniformly thick over the whole surface. Here and there, arranged sometimes at random but more often in some remarkably uniform pattern, there occur thin places in the wall where secondary deposition has never taken place. A further remarkable thing is that wherever one cell has such a thin place, the adjacent cell in contact with it has a similar thin place at the corresponding point in the wall. These are spoken of as *pit* pairs. In parenchyma cells this leads to the development of cylindrical canals in the wall which are closed off merely by the two primary walls of each cell and the tenuous middle lamella between them—the *simple pits* (Fig. 4). Such pits have a most profound influence on the structure of the wall area immediately adjacent to them. Of even more consequence are the *bordered pits* typical of, for instance, the tracheids of conifer stems. Here, as the secondary wall becomes thicker

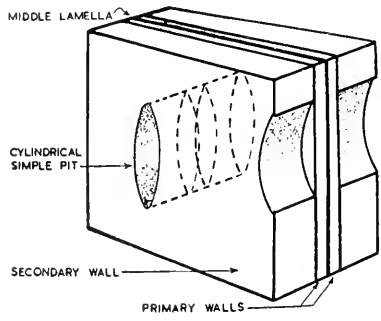

FIG. 4. Part of the walls of two adjacent parenchyma cells showing the form of simple pits. The section at the right of the figure shows that the pit membrane is three layered. No attempt is made to show protoplasmic connections.

by the continual depositions of new layers within the old, the new layers encroach over the thin region so that this becomes over-arched and, by the time that secondary deposition ceases, the "hole" in the wall has become much smaller (Fig. 5). This leaves scattered over the wall a series of over-arched regions within which, and to some less extent surrounding which, the wall structure is quite different from elsewhere. This is of particular importance in, for instance, the walls of the vessels in *Quercus alba* and in many other ring-porous dicotyledonous trees (see Fig. 53), where the pitting is crowded over almost the whole wall surface and is bordered, narrowly in contact with ray cells and wood parenchyma and more widely in contact with other vessels.

FIG. 5. For explanation, see text. (Reproduced by permission from *A Textbook of General Botany* by Holman and Robbins. Published by John Wiley & Sons, Inc., 1938.)

There can be no wonder, then, that study of structural details in botanical material by physical methods is fraught with difficulties and beset with pitfalls. Although the bulk of the material which the biophysicist is called upon to handle here is composed of secondary walls and can therefore be examined in dead tissue, the morphological complexities alone are still so enormous that progress is of necessity rather slow. Naturally, therefore, it is desirable to begin with the simplest possible case. To those who are interested primarily in the more academic aspects of plant behaviour, this would lead naturally to a study of those cells which grow unimpeded by the obstacles to growth, or the control of growth, consequent on tissue formation, *e.g.* to the algae where the reactions of cells could be investigated independently. Historically, however, the cells which took precedence in the modern investigations of submicroscopic structure in plant cells were the phloem fibres, and in particular ramie fibres, for several reasons, not the least of which is the economic importance of such cells. These are, in fact, rather satisfactory, since they are composed of cells all of one type and approximately of the same size; long thin cells, some one hundred or more times longer than wide, with long, tapering ends and with thick cell walls. These fibres are, moreover, arranged quite parallel to each other, or are so long that cells separated chemically from the tissue can be laid strictly parallel to form a bundle, the properties of which reflect

largely the properties of a single cell. This, together with other simplifications inherent in fibre structure, which will appear later, enables the fundamental structural features of the material constituting the walls of plant cells to be determined with some certainty. Investigation of structure in other cell types and in more complicated tissues then resolves itself into the application of the knowledge thus obtained to the more complex forms. Even with the fibres, however, there is the complexity that the cells are not strictly the continuous hollow cylinders into which physicists, for the best of reasons, prefer to sublimate their ideas. The long, tapering ends scattered up and down any naturally occurring bundle of fibres introduce some degree of uncertainty into the orientation of the constituent cell walls. As far as the investigation of the fundamental features associated with the crystallinity of the walls is concerned, this does not have any important effect; but it cannot be ignored when attempting completely to delineate the properties of any single cell from the properties of a bundle.

CHAPTER III

The Chemical Nature of the Constituents of the Secondary Wall

BOTH THE primary and the secondary walls, whose formation has thus been briefly considered, are built up from a wide variety of constituents, into whose chemical nature some further inquiry is essential before proceeding to the physical aspects of their organization with which this book is mostly concerned. The basic constituent of the walls of cells in plants, with the particular exception of the fungi and of some algae, is the polysaccharide cellulose and it is natural therefore that discussion throughout the whole of the following pages will centre largely round this substance. With the exception perhaps of the proteins, there is no other substance produced by living things which has received so much attention, and about which so much is known. This is primarily because cellulose occupies such a prominent place in human economy; but naturally details of structure are also of paramount importance to an understanding of cell growth. Nevertheless there are many other substances associated with the skeletal cellulose which are not without considerable interest and which can modify profoundly the properties of the whole wall and therefore the nature of the cells concerned. It is difficult, if not impossible, to characterize chemically these "incrusting substances"* but they may be said with some justification to fall into three main groups. Whereas cellulose on hydrolysis yields glucose only, there are a number of wall constituents which yield either a different sugar or a sugar derivative and these have been collectively given the unfortunate name *hemicelluloses*—unfortunate since in structure and function they are quite distinct from cellulose proper. Taking the latter group first, there is a large number of substances which yield a sugar acid (a glucuronic acid whose nature will be discussed later); these are much more labile than the cellulose and sometimes act as a food store, appearing in the wall and disappearing again during the metabolic processes associated, for instance, with seed

* This term is used to imply that the structural substance in the wall is cellulose, and that these other substances are deposited within it in such a way that the properties of the wall are modified in degree but not in kind.

development and germination. Strictly speaking, one of the most widely occurring non-cellulosic wall materials—*pectin*—should be included in this group since this substance also yields a sugar acid on hydrolysis; and it seems safe to say that, with the possible exception of the fungi, there is no plant known which does not contain pectic substances. In the former group, on the other hand, there are a number of substances which resemble in general molecular morphology the ubiquitous cellulose, are closely associated with it, and are therefore extracted from cellulosic bodies only with difficulty. These are referred to as *cellulosans*, All these substances occur in both primary and secondary walls and, in particular, are undoubtedly present in the former during growth (see Table I). *Lignin*, however, a substance whose composition is as yet incompletely known, becomes prominent only as the cell ceases to be metabolically active and in many cases its development precedes the death of the protoplast. It is therefore associated in tissues largely with mature or dead elements, notably the cells of xylem or wood (Table I) and the fibres of the phloem. It is prominently present in wood parenchyma cells, which remain alive for long periods after its deposition. All these substances are naturally of very considerable importance both academically and in technology, so that there is a very extensive literature, much of which is readily available. It is proposed here, therefore, to give only the briefest sketch of this aspect of wall chemistry; for further information reference may be made to any one of a range of excellent text-books (3). Since cellulose itself occupies the prime place in any discussion of wall investigation, its chemical nature is the first to come under review.

Cellulose

It has been recognized now for more than a hundred years that cellulose on acid hydrolysis yields large quantities of glucose, and it is, in fact, now nearly thirty years since it was first established that the yield of glucose is almost quantitative. This completely insoluble and comparatively inactive substance must therefore be composed almost exclusively of a complex of the soluble monosaccharide. The conception of the synthesis of small molecular species into large molecular combinations with completely different properties is, of course, now a commonplace in this age of plastics; and it is perhaps redundant to point out the implication that the glucose units in cellulose must be linked in such a way that many of the —OH groups are to a large extent protected against the approach of water molecules, in order to confer insolubility. This is achieved by a now familiar trick of

TABLE I

The composition of the cell walls in some plant tissues, after alcohol-benzene extraction (all figures expressed as % dry wt.)

Material	*Cellulose* 1	*Lignin* 2	*Pectin* 3	*Protein* 4	*Pentosan in cellulose as % in cellulose* 5	*Polyuronides not extracted by amm. oxalate* 6	*Authority* 7
Cambium							
Ash	20·2	4·6	21·6	29·4	26·9	4·9	Allsopp
Elm	20·7	13·5	9·9	30·0	26·3	4·6	
Pine	25·1	8·6	16·6	20·8	36·9*	4·0	and
Sapwood							Misra
Ash	58·3	20·9	1·58	1·37	24·5	4·1	
Elm	57·5	24·8	1·68	1·73	23·8	5·3	(1940)
Pine	61·8	26·1	0·96	0·83	14·1†	—	
Rye grass	58	20·0	—	—	10	—	Recalculated from Norman and Richardson (1938)
Bamboo fibres	60·8	31·4	Estimated with 6	5·0	22·2	4·6‡	Singh (1949)
Jute fibres (good Tossa)	74·4	10·37	—	—	12·04	—	
Flax (Indian)	91·2	3·27	—	—	3·01	—	
Hemp (Indian)	77·27	7·28	—	—	1·79	—	Norman (1936)
Manilla hemp	74·14	8·51	—	—	14·01	—	
Coir	55·69	30·59	—	—	13·21	—	
Oat Coleoptiles	42	—	8	12	—	38	Thimann and Bonner

* 21·9 of this is mannan the rest of the figures refer to xylan alone.
† 6·2 of this is mannan the rest of the figures refer to xylan alone.
‡ *Total* polyuronides.

nature, a trick which is repeated again and again—in the proteins, for instance, as well as with other polysaccharides—and which man is now at last learning to copy and even to improve upon. A clue to the kind of association involved in cellulose was already at hand in the appreciation that under certain conditions a disaccharide, cellobiose, can be detected amongst the products of hydrolysis, for it could readily be assumed that the glucose

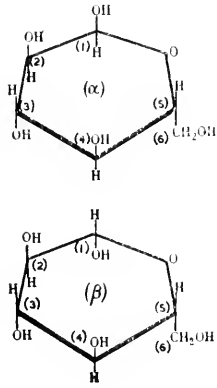

FIG. 6. Diagrammatic representation of the stereochemical formulae for the two types of glucose in the 6-membered ring (pyranose) form. As usual, the corners of the ring are occupied by one carbon atom each, which is not shown, with the exception of that carrying oxygen. The carbon atoms are numbered in the figure following the usual convention. Carbon 1 carries the potentially reducing group. The rings are drawn in perspective and the upper of each pair of radicles attached to a carbon is supposed to lie above the plane of the ring, and the other one below this plane. *Upper Figure.* The formula of α-glucose. This does not occur in cellulose but does form the basis of the starch molecule. *Lower Figure.* β-glucose, differing from α-glucose only in the relative positions of the —H and —OH attached to carbon 1.

pre-existed already in the cellulose in the form of cellobiose residues. This has indeed turned out to be the case. Following the elegant work of Haworth and his collaborators, it is known that the glucose units in cellulose occur in the six-ringed modification and exclusively in the β-form (Fig. 6). Since the reducing power of cellobiose

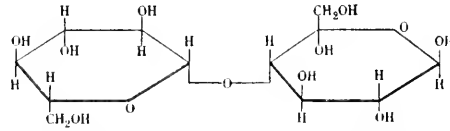

FIG. 7. *Cellobiose.* The molecule could obviously be increased in size by further condensation, on each end of the cellobiose molecule, of the other glucose units. The resulting long-chain compound would be cellulose.

is doubled on hydrolysis then it follows that the union between the two constituent glucose residues must be through the potentially reducing group of one of them (carbon 1 in Fig. 6) and it remains to determine to which of the four possible non-reducing groups on the other sugar molecule this union is made. This has been done by chemical methods into which we need not go here (4) and it has turned out that the attachment is to carbon 4, giving the 1:4 or glucosidic link between the two glucoses (Fig. 7). It will be noticed that this link is made by the separation from the two glucose molecules of the elements

of water, and it is for this reason that the two units in cellobiose may be referred to as glucose residues. As an obvious extension of this idea of a 1:4 link, and in consequence of the fact that in cellulose only three —OH groups per glucose residue are available for nitration or acetylation, it follows that the two outer ends of each cellobiose residue in cellulose may be in turn joined by 1:4 linkages to other cellobiose residues. Thus the chemical evidence leads directly to the conception in cellulose of long chain molecules in which the constituent links are β-glucose residues. This is almost as far, however, as direct chemical evidence goes and it leaves open a number of questions which it is most imperative to answer in terms of such a model of cellulose structure. How long are these chains? How are they aggregated together? Are they pointing in definite directions or are they arranged at random? These are questions which cannot be answered at all, or only by inference, by purely chemical methods and further discussion must be postponed until suitable physical methods of approach have been described.

Hemicelluloses and pectic substances

The hemicelluloses, as has been pointed out already, form a complex mixture of substances which have not yet been clearly defined either theoretically or analytically, but in general they are more readily hydrolyzed by acids, and more soluble in alkalis, than is cellulose. The amount of hemicelluloses present in cell walls varies considerably from a very small percentage in cotton hairs, for instance, to something over 50% in some collenchyma cells. It is quite clear that even now, after many years' continuous attention, it is impossible completely to solve the question of the constitution of these substances—it is not even possible to say whether the different sugars available from their hydrolysis arise from the same molecular species or not—but for present purposes they may be classed as two main groups, the *polyuronides*, including the *pectic substances*, and the *cellulosans*.

The polyuronides

Corresponding to the polysaccharides, the polyuronides consist apparently of chains of sugar residues in which the —CH_2OH groups are replaced by —COOH groups, *i.e.* of uronic acid residues. A number of different compounds of this kind have been detected in plants (3) but the commonest seems to be the one associated in pectic substances. Here the uronic acid is probably polygalacturonic acid, in some close association with galactose and arabinose, which led Ehrlich and other earlier workers to assume a ring formula (see *e.g.* 5) consisting of four

galacturonic acid residues connected through one of galactose and one of arabinose. Such attempts to formulate a structural formula are, however, of historical importance only and need not be dwelt upon. It seems now clear, through close examination of chemical as well as physical properties, that the structure based on polygalacturonic acid (Fig. 8) proposed by Meyer and Mark in 1930(6) is in essence correct, though chemical evidence for the type of linkage (whether, for instance, it is the 1:4 link as in cellulose and as illustrated in Fig. 8) is lacking. It seems safe, therefore, to regard pectic acid as a long chain polymer, and pectin itself would then be the almost completely methylated

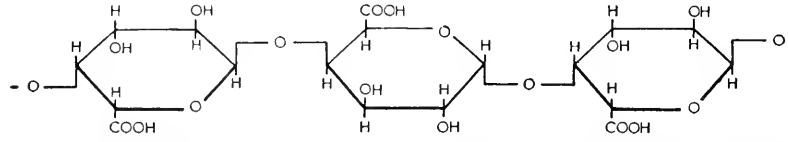

FIG. 8. *Polygalacturonic acid.* Three glucuronic acid residues are shown linked by 1 : 4 primary valences. The chain could obviously be continued indefinitely at either end.

product. Pectin occurs in the walls of almost all plants and forms the middle lamella in growing tissues. A jelly-like substance such as this is obviously very suitable for maintaining cells in close proximity to each other, while allowing mutual displacements, and is therefore very suited to conditions in the growing points. In adult tissues, however, the cells must be cemented together very firmly, and it is of interest to note that in these adult tissues the middle lamella is often converted to calcium pectate, forming a hard cement soluble in 0·5% ammonium oxalate only after treatment with acid (*e.g.* by a mixture of equal parts of alcohol and hydrochloric acid in the cold for twelve hours or so). This would appear to involve the conversion of calcium pectate into pectic acid (which, incidentally, occurs in nature only as the calcium salt) and its subsequent solution in the oxalate. A third type of pectic substance, or probably class of substances, is given the name of protopectin. This, too, forms a hard cement, but its relative insolubility is probably due rather to a close association with other wall substances than to any special feature of its molecular composition.

Cellulosans

The cellulosans, on the other hand, form a distinct group of substances distinguishable from cellulose in that they yield pentose sugars on hydrolysis, and from the polyuronides in that they do not yield uronic acids. The chemical and physical evidence which will be

discussed later on suggests that there is a close association between the cellulosans and cellulose (7), though there are some aspects of more modern work which make it clear that the kind of association involved is by no means so certain as was at one time thought. This, however, will be a subject for inquiry later on when the general picture of wall organization has been painted in.

Among these substances, two are very widespread. *Xylan*, a polymerized xylose, is universal in the Angiosperms, and *Mannan*, giving mannose on hydrolysis, is characteristic of the Gymnosperms. This is a most remarkable distribution of a chemical substance and must, presumably, be of some significance, though no particular attention has hitherto been devoted to its implications. Both are extractable with difficulty from cellulose, either acid hydrolysis—which is liable also to attack the cellulose itself—or treatment with 5% caustic soda being necessary for their removal.

The cell walls of plants thus constitute a most complicated mixture of a number of sugars joined together to form molecular chains, whereby soluble and reactive units become linked into insoluble and less reactive bodies, and it is therefore a somewhat difficult task to reveal in its entirety the detailed organization of the wall. The problems involved are rendered even more difficult when, as so often happens in dead tissue and more particularly in those tissues most easily handled, the wall becomes impregnated also with other substances of a non-sugar type. Of these undoubtedly the most common, and the only one with which it is necessary to deal here, is lignin.

Lignin

The chemical nature of lignin is as yet unknown and the many structural formulae which have been proposed as representing its constitution (8) alone emphasize the dearth of concrete facts. It was, in fact, at one time considered that lignin might be a decomposition product of the saccharides in the wall consequent upon attempts to achieve its isolation! The difficulties involved in its isolation are certainly very considerable, and it is in this that the obstacles in the way of a final elucidation of its structure are to be found; but there can be no doubt now but that lignin does represent a complex of substances which are actually present in the wall. Of the various attempts which have been made to define the substance, perhaps the most convincing and certainly the one in most complete harmony with the chemical data is that proposed by Freudenberg (9). According to his view, lignin is built up from aromatic nuclei such as I, II and III (Fig. 9) to which may be

attached the side chains IV, V or VI. It appears further that in the lignin from Angiosperms only III is present, while in Gymnosperms I and II are involved, demonstrating yet another chemical difference between these two large groups of plants. The structures shown in Fig. 9 are,

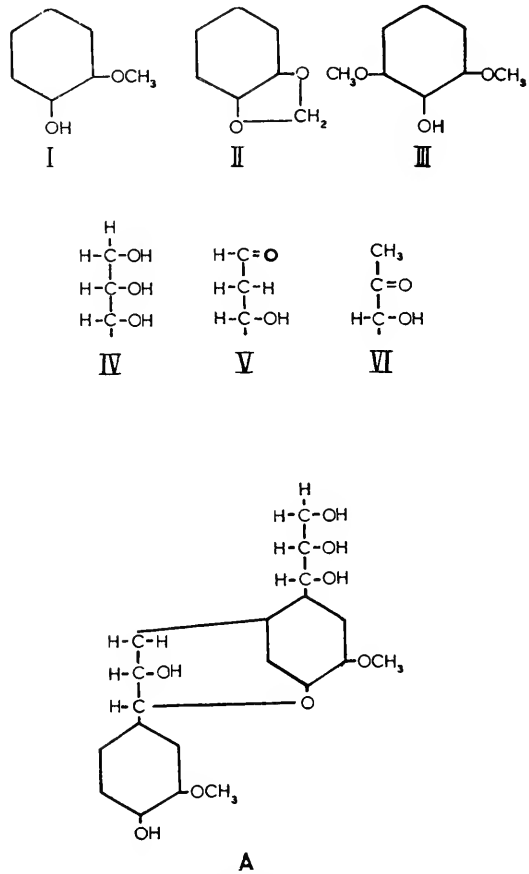

FIG. 9. For explanation, see text.

of course, only the units of which lignin is composed, and it remains still to be settled in what way these units are linked together. Freudenberg himself suggests that lignin in the wall may be considered as a product resulting from the etherification and subsequent condensation of the above units, to produce complexes such as *A* in Fig. 9. These, and the corresponding products from II and V, III and VI, are supposed to undergo further etherification and condensation by means of which

extremely complicated molecules of high molecular weight might be built up.

The physical properties of lignin are also consistent with such a view of its constitution. Thus the high refractive index (1·61) is considered to be in harmony with the proposed structures, and the ultra-violet absorption maximum at 2800 Å. is in accord with the absorption spectrum of known aromatic compounds. While the molecular weight of lignin is therefore probably high, the figures recorded in the literature for extracted lignin are rather low, the value depending on the technique of isolation and upon the method of molecular weight determination. Using the ultra-centrifuge Gralen (10), for instance, has recorded molecular weights as low as 3500, though in other instances the figure turns out to be of the order of 40,000. On the whole, therefore, the evidence suggests that lignin does not form long chain molecules like cellulose and the other polysaccharide derivatives in the wall, and it is understandable why earlier attempts to find a precursor of lignin among, for instance, the pectic substances have led to such dismal failures.

Staining reactions

So much, then, for the chemical nature of the material whose physical attributes are to be studied in the following pages. One last word may, perhaps, be said about the common methods of detecting the presence of the various substances concerned since these will be referred to again and again in this book. None of the staining reactions for the incrusting substances can be said to be specific, but if applied with care and with due recognition of the dangers involved, and particularly if considered in conjunction with specific solubility tests, they can be used with some degree of certainty.

Taking cellulose again first, the blue coloration with iodine followed by 70% (by weight) of sulphuric acid is generally accepted as a certain test of its presence.* The absence of a blue coloration is not, however, any guarantee that cellulose is absent, for a chip of wood gives no cellulose reaction although perhaps as much as 60% of its dry weight is made up of this polysaccharide. Only after lignin removal is a positive reaction obtained, so that with negative responses to this test due consideration has to be given to the possible masking effects of other substances. Among certain species of the algae a positive test for cellulose is obtained only with the greatest difficulty for other reasons which will appear later on. Cellulose can be further characterized among substances liable to be present in cell walls by its ready solubility in

* There are, however, prominent exceptions which will not be discussed here.

cuprammonium (Schweitzer's reagent) (though again this fails in some algae) and by its transparency to ultra-violet radiation. Undoubtedly the surest indications of the presence of cellulose are, however, those associated with its crystalline nature. These will be discussed at some length in the next chapter.

Pectin can be recognized by the deep blue coloration in methylene blue, and the red coloration on prolonged immersion in aqueous ruthenium red (1/10,000). These reactions are, however, given by other substances present in some cases (oxidized cellulose, cytoplasmic debris, etc.), and it is imperative therefore in all cases of doubt to check on the results by solubility tests. Pectin itself is soluble in 0·5% ammonium oxalate and is reprecipitated in alcohol; if other pectic derivatives are involved it may be necessary to apply a preliminary hydrolysis in, for instance, alcoholic hydrochloric acid. The presence of xylan or mannan can be demonstrated only by hydrolysis followed by a test for the corresponding monosaccharide.

There are several characteristic colour tests for lignin, but again these suffer from lack of absolute specificity. The red coloration in phloroglucin followed by concentrated hydrochloric acid is a test for pentosans rather than for the particular groupings present in lignin, and the same probably applies to the yellow reaction in aniline chloride. On the other hand, the magenta coloration when the material is treated with chlorine water (or its equivalent) and subsequently heated in 3% sodium sulphite can be regarded as typical, though the colour developed depends upon the material used, being quite different in, for instance, the Angiosperms and the Gymnosperms; and the silver staining method recently introduced by Coppick and Fowler (11) promises to be of considerable value. Lignin can be extracted from walls by the former of these two treatments but during the process very considerable degradation occurs. In fact, there cannot be said to be any true solvent for lignin, and the substance is therefore usually isolated by removal of the other accompanying substances.

With this brief description the way is now open for the study of the complex of these various substances by the physical methods to be described. Before proceeding to the study proper, a chapter will be devoted to a simple account of these physical methods in order to confer upon the reader some ability critically to assess the value of the work to be described.

CHAPTER IV

Physical Methods for the Investigation of Structure in Plant Cell Walls

IT SHOULD be clear from the preceding chapters that the cell walls of plants owe their peculiar properties to the presence of cellulose, so that investigations of structure in these walls is to a large extent an investigation of the structure of this substance. That is not to say, of course, that the incrusting substances can be ignored. Far from it, for they do modify to a considerable extent the features of cell walls which would, in the absence of these substances, be determined only by the cellulose itself. Nevertheless, since cellulose forms the framework of the wall, so that the incrusting substances can be removed without causing any loss of form in the cell, then it must receive first attention. Fortunately, therefore, cellulose is so constituted that it is, in a molecular sense, crystalline and confers upon cell walls some crystalline properties. This is at first sight rather surprising, since cell walls never show the beautiful external features, such as crystal faces, which we normally associate with the crystalline state. It is nowadays, however, almost axiomatic that a substance can possess internal crystallinity without showing the external form of crystals.

Since cellulose occurs in such a crystalline state, it becomes imperative to consider for a little while just what is implied by the term *crystalline*, and the methods whereby the nature of crystalline material can be investigated. Attention will be confined here to two methods only—the method of X-ray crystallography and the method of polarization optics. These are undoubtedly the two most important tools available for wall studies and, used under properly controlled conditions, they can together present a rather complete picture of the organization of these walls. It is not possible in the space available to give anything but a brief elementary discussion of these methods, and those readers who wish to go further into these matters should consult any one of a range of text-books(12). In particular, attention is here confined largely to the crystal system to which cellulose is thought to belong, to the exclusion of all other crystal types, and the examples used are, for

reasons of simplicity of later expression, confined to cellulosic materials alone.

Crystal lattices

It is the very essence of a crystal that its molecules shall be arranged in some very regular way, and it is naturally the task of the crystallographer in the first place to define this regularity. The first question is, therefore, how much is needed to be known to achieve this end? Consider first a very simple case of a series of points arranged in a straight line, ignoring for the time being the type of atom, or atom group, associated with each point but assuming that each point is identical with any other in this respect. Then if the points are arranged regularly, exactly the same distance apart, this arrangement could be regarded as a "linear" crystal or a one-dimensional lattice (Fig. 10(*a*)). Knowledge, therefore, of *a*, the distance apart of the points, is all that is needed to define the system completely. One parameter defines the lattice. If, now, a number of such lines of points are placed parallel to each other (Fig. 10(*b*)), again regularly the same distance apart, and bearing some constant positional arrangement to each other such as that shown in the figure, then three parameters are necessary to define the new, two-dimensional lattice—*a*, and *b*, the distances apart of the points along two different directions, and the angle between these two parameters. These define a parallelogram *ABEF* such that by the regular placing of other identical parallelograms on each side of *ABEF*, and continuing this process indefinitely throughout the plane, then the whole "crystal" can be built up. Now, however, these are not the *only* three parameters which could be chosen to define the structure; a parallelogram such as *CDFG* could be used with exactly the same final result, and there is obviously, in fact, an infinite number of parallelograms which could arbitrarily be chosen. The only reason for preferring *ABEF* would be its simplicity.

If, now, sheets of points as in Fig. 10(*b*) are arranged parallel to each other, one over the other and arranged regularly the same distance apart (Fig. 10(*c*)), then in general six parameters are needed to define the lattice completely and the labour involved is consequently considerably increased. Fortunately, in the monoclinic crystal class to which cellulose belongs, the number is reduced to four, since $\alpha=\gamma=90°$. Here again it should be noted that though the parameters marked on the figure delineate a parallelepiped *ABEFLMOP*, regular repetition of which throughout space will reproduce the system, this again is not the only parallelepiped which will satisfy this condition, and

again it may be chosen rather than any other solely on the grounds of simplicity. Such a body is called a *unit cell.*

Before proceeding to consider the methods by which these parameters can be determined and to observe the conclusions which can then be

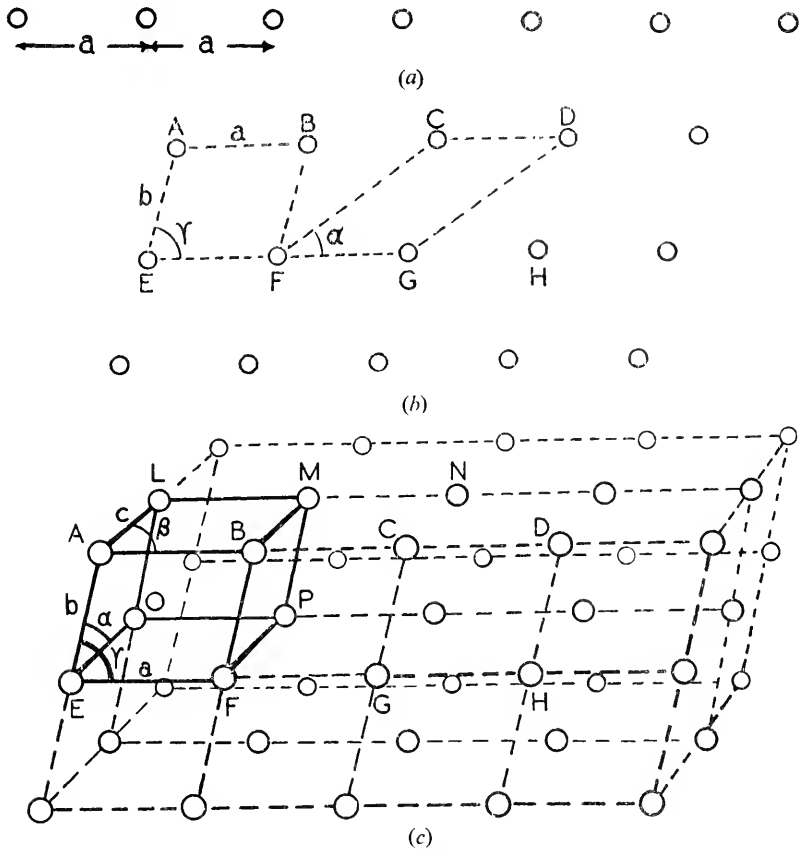

FIG. 10. For explanation see text.

drawn, it will be profitable to consider some other features associated with this regular repetition of points in space. Returning for the moment to the two-dimensional lattice (Fig. 10(*b*)), it will be noticed that not only do the points lie along the lines by which their position was defined above, but they lie also along other lines such as *IB*, *JC*, *KD*, etc. (Fig. 11); in fact each point is the meeting-place of an infinite number of different lines all running through these points. Suppose it is necessary to define any one of these sets of parallel lines in terms of

those defining the parallelogram *ABEF*, say the set *IB*, *JC*, *KD*, etc. (Fig. 11). Then this could be done by, for instance, stating the angle ϕ. An alternative way, and the way always adopted, seems at first sight more cumbersome. If we start from any point *I* as origin then, on passing along the lattice in the *a* direction, two lines are crossed before reaching the next identical point. The lines can therefore be allocated the number 2 in the *a* direction. On passing in the *b* direction only one line is passed through and the corresponding number is 1. The sets of lines *IB*, *JC*, *KD* can therefore be defined as the 21* lines, and their distance apart can be calculated. These numbers are referred to as the *indices* of the lines, and a further example, the 11* lines, is given in Fig. 11. Conversely, if the distance apart of a number of lines is known, together with their indices, then the dimensions *a* and *b* can obviously be calculated. It should be noted that the indices of a line can be found alternatively by noting what fraction of the parameter distance lies between each set of two neighbouring lines. Thus for the lines *IB*, *JC*, etc., the intercepts are $a/2$, $b/1$, corresponding to the indices 21. Each index represents therefore the reciprocal of the relative intercept of the corresponding parameter cut off by two neighbouring lines. In three dimensions the same convention is applicable, but now three indices are required instead of two, and an example is presented in Fig. 12, representing the planes 112. Planes parallel to one edge of the unit cell will have index 0 for the corresponding direction, so that the faces of the unit cell can be represented by the indices 100, 010, 001. What is needed, therefore, to define the spatial arrangement of the whole lattice

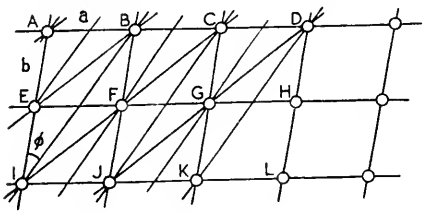

FIG. 11. For explanation see text.

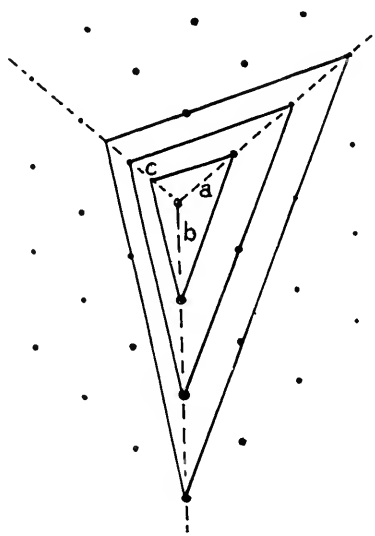

FIG. 12. For explanation see text.

* Formally, the last figure should be written $\bar{1}$, implying that the index is -1, since the first line to the right of *I* cuts the *b* axis below, and therefore on the negative side of *I*.

is merely the mutual arrangement and the distance apart of the three sets of planes 100, 010 and 001, and these will need the six parameters mentioned above, or four in the case of the monoclinic system to which cellulose belongs. Just as in the case of the plane lattice, so here these parameters can, under certain conditions, be determined from the positions and distances apart of other sets of planes, provided these can be determined in sufficient detail. Naturally, the more numerous the sets of planes available the more accurately can the unit cell be determined. When, however, this desirable end has been reached, this forms only the beginning. All that has been learned is the form and dimensions of a set of points, and for a full description of the crystal it is then necessary to determine what atom or atom group is associated with one such point and how the atoms are arranged round the point. In general this much more difficult determination cannot be achieved by physical methods alone, and in the case of the more complicated crystals cannot at present be achieved at all with any great precision. So with cellulose, recourse has to be made, once the unit cell dimensions have been determined, to the body of chemical evidence available and to other physical data, in order to set the constituent glucose residues in approximately their correct relative positions.

The first problem, then, in attempting to elucidate the structure of cellulose is to determine the unit cell, and a few pages will be devoted to the techniques involved. These are, of course, the techniques of X-ray analysis suitably modified to deal with the peculiarities of biological material, and it will not be possible to present anything like a full and logical account of the method. Enough may perhaps be said to give the reader a general idea of the processes of thought involved. It is further to be noted, however, that in many crystals the regular arrangement of the atoms and molecules within them confers upon them special features which can be investigated by methods other than those of X-ray analysis, and one of these—anisotropy of optical properties—presents in cellulose a particularly valuable tool. In some ways the polarizing microscope is complementary to the X-ray spectrometer, as will be seen when the results obtained by the two are later compared. In addition, the polarizing microscope is merely a modified form of the common tool of biologists, and therefore investigations with its aid need no very special apparatus and no serious problems of maintenance; though this is not to say that the method can be used by untrained workers without the risk of very serious misinterpretation. Both the methods of X-ray analysis and of polarization optics will therefore be reviewed very briefly before passing on to the results.

X-ray analysis of plant cells

It is taken for granted that readers are familiar with the conception of radiations of all kinds, including light and X-ray radiation, as a series of vibrations transverse to the direction of propagation. Classically, to overcome the difficulty that light will pass through a vacuum, the concept was introduced of an "ether", an imponderable substance which permeated all material bodies throughout space and whose constituent particles were thrown into vibration by the passage of light—whose vibrations *were* in fact the passage of light. In this sense, therefore, radiation can be considered to have two "directions", a direction of propagation and a direction of vibration at right angles to it.

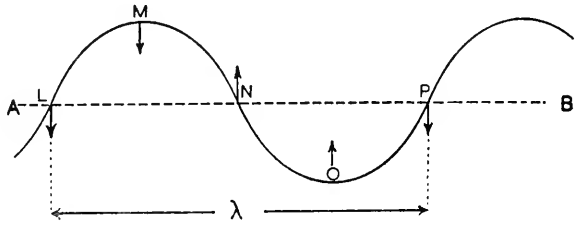

FIG. 13. For explanation see text.

Suppose, therefore, as in Fig. 13, a beam of radiation is passing from *A* to *B* and the condition of the vibration is observed instantaneously. The trace of the positions of successive "particles" will be a series of sine waves *LMNOP*, etc. A particle at *L* is momentarily undisplaced, but is on the point of passing downwards. Particles such as *M* are momentarily at rest but are on the point of moving downwards, etc. Clearly at whatever moment we "stop" the vibrations for inspection the distance *LP* will be the same; this is then a characteristic of the vibration and is called the *wavelength* symbolized by the Greek letter λ. There are, naturally, other ways of characterizing any beam of radiation, but this one is most suited to present purposes. Light beams of different colours, for instance, differ in wavelength from 0·4 μ (blue) to about 0·7 μ (red). X-rays differ from both by their very much shorter wavelength, which is never more than a few Ångstroms* at most, and it is to these radiations of very short wavelength that attention will for the moment be confined.

It is further taken that the reader is acquainted with the use of line gratings in optics to produce diffracted beams of visible light—the use of gratings to produce a spectrum, for example. Fundamentally, the same principle is applied in the use of X-rays here with the difference

* One Ångstrom (Å.) is 10^{-8} cm.

that the wavelength of the radiation is much shorter, since the distance between the diffracting points is much less. For the great bulk of the work on cellulose an X-ray wavelength of 1·54 Å. is employed as given from copper bombarded with electrons at, say, 40,000 volts. Space will not allow any reference here to the experimental methods used to produce X-rays; reference must again be made to other texts (12, 8).

Returning to the line lattice, suppose a beam of X-radiation is allowed to fall on the lattice in the direction defined by θ_1 (Fig. 14). Most of the radiation will pass straight through the specimen. Since, however, each point on the lattice can be regarded as a point of secondary emission, then there is a possibility of radiation in other directions. In fact,

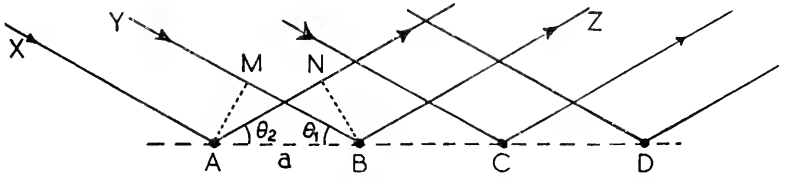

FIG. 14. *Reradiation by a row of points.* Radiation is incident on the row from the left at angle θ_1. Consider any direction on the right making an angle θ_2 to the row as shown. Take any two neighbouring points, *A* and *B*, and drop perpendiculars *AM* and *BN* as shown. Then *A* and *M* are "in step" and the perpendicular *AM* can be taken to represent say a crest in the radiation. For reflection to occur at the angle θ_2, *N* and *B* must also be "in step", so that

$$MB-AN=n\lambda,$$

where $n=0, 1, 2, 3 \ldots$ *i.e.*

$$a\cos\theta_1-a\cos\theta_2=n\lambda,$$

for $n=0$, $\theta_1=\theta_2$, *i.e.* a "reflection" occurs where the angle of incidence equals the angle of reflection. Other reflections can be found for $n=1, 2, 3$, etc., but these progressively diminish in intensity.

radiation will not be emitted in any particular direction only if the individual radiations from each point cancel out, and this will normally happen since a crest of the radiation from one point will meet a trough from some other. The construction in the diagram shows, however, that in one direction, making the same angle with the row of points as the incident rays, reflection will in fact occur, *i.e.* the radiation can be regarded geometrically as being reflected from the line of points. This is the condition when the total path of the rays from any one point is of the same length as that from a neighbouring point. Equally, of course, there will be a "reflection" if the two path lengths differ by one wavelength or, in fact, any whole number of wavelengths. There are, therefore, a number of "reflections", decreasing in intensity as the path difference increases from 0 to 1λ, 2λ, etc. Each of these "reflections" is not, of course, a single ray, but rather a cone of rays making the same

angle with the line of points. If, however, the line of points is now regarded as the intersection with the paper of a plane of points lying at right angles to the page surface, then it can be shown quite simply (see *e.g.* 13) that a beam of X-radiation falling on the sheet, as represented in Fig. 14, will have reflected *beams* as shown. Ignoring higher orders (with path differences greater than zero), then any parallel beam of radiation will produce a reflected beam whose angle of reflection is equal to the angle of incidence.

Turning now, therefore, to the three-dimensional lattice, the same

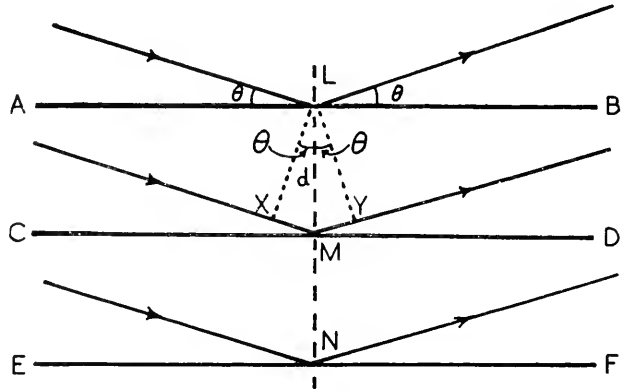

FIG. 15. *AB*, *CD*, etc., represent loci, in the plane of the paper, of molecular planes spaced *d* Å. apart and standing normal to the plane of the page. The construction shows that the ray reflected by the plane *CD* has a path difference greater than that reflected by *AB* by an amount

$$XM+MY=2d\sin\theta.$$

Hence reflection from successive planes will fortify each other if

$$2d\sin\theta=n\lambda,$$

n being an integer.

conditions must apply; except that now account has to be taken of the interference between "reflections" from neighbouring planes. Clearly, there will always be a finite path difference between the reflections from one plane and the next; if this difference is a whole number of wavelengths then reflections from the planes will fortify each other and reflection will occur. Again, the strongest reflection will be the first order, when the difference is one wavelength, and under these circumstances it will be clear from Fig. 15 that the wavelength λ, the reflection angle θ and the interplanar spacing d are related in the form

$$\lambda=2d\sin\theta, \qquad ..(1)$$

first derived by the Braggs and known as the Bragg Law. If, therefore, λ is known and θ can be measured, then the value of d can be calculated.

It should be noted that the angle between the primary beam and the first order reflection is 2θ.

Suppose, therefore, that a crystal is set up in the path of a parallel beam of X-rays and a photographic plate is placed some distance D behind it (Fig. 16), then the position of the primary beam will be recorded as a spot on the plate. This is usually so intense as to cause serious fogging of a large area of plate, so that usually a small lead cup is inserted to intercept the beam, as in Fig. 16. The first order reflection will be recorded at R, say, so that the distance $RO=r$ can be measured. The value of θ can then be calculated from the relation

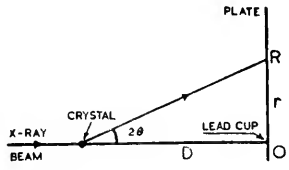

FIG. 16. For explanation, see text.

$$r/D=\tan 2\theta. \qquad ..(2)$$

Substitution of this value of θ in equation (1) will then give the corresponding value of d, the interplanar spacing responsible for the reflection. In general, of course, the crystal will not be in such a position as to give any particular reflection, but rotation around an axis normal to the beam will cause the reflection to appear; further rotation until the same reflection occurs on the other side of the central spot makes for more accurate measurement since the value of $2r$ can then be determined without attempting to define the position of the primary beam.

This in brief, then, is the method used to determine a whole series of interplanar spacings. If, now, these reflections can be "indexed", *i.e.* if the indices of the planes producing the reflections can be found, then the validity of any proposed unit cell can be tested. From this point of view it should be noted that, though for simplicity attention has been confined so far to first order reflections, in the photographs to be discussed second and even third order reflections occur and this is liable to lead to confusion. For this reason it has become standard to include the order of the reflection in the index. Thus, if we take for simplicity planes parallel to the basal plane of the unit cell (Fig. 21), then reflection occurs from the 010 planes as a first order reflection when there is a path difference of one wavelength between waves from successive planes spaced b Å. apart. The second order reflection from these planes, when there is a path difference of two wavelengths between successive planes, can be considered as first order reflections from planes spaced half the distance b apart, *i.e.* from planes with index 020, and the reflection can be so recorded. Similarly, for any combination of indices, the third order reflection from planes with indices 211, for instance, can

be regarded as a first order of planes indexed 633. This leads to a third meaning of the indices of planes defined in this way, a meaning which will be needed later on. All "reflections" are first order reflections from planes defined in the above way, and there is a path difference of one wavelength between successive planes. Counting the number of planes crossed between one lattice point and the next, therefore, is the same thing as counting the number of wavelengths path difference between waves scattered by neighbouring lattice points. The indices thus represent the phase difference between waves diffracted by neighbouring lattice points along each of the three axial directions.

The rotation diagram

Turning now to the special features associated with the study of plant material, it will be seen later that the biophysicist has to deal with photographs closely resembling those given by a single crystal continuously rotated about an axis perpendicular to the X-ray beam, the so-called *rotation photograph*. A few moments' consideration may, therefore, be given to the particular features involved. Suppose a crystal is set up in an X-ray spectrometer in such a way that a parallel beam of X-rays falls on the crystal at right angles to one of the crystallographic axes (*i.e.* one edge, say the b edge, of the unit cell), and let A and B (Fig. 17) be two neighbouring identical points of the lattice, *i.e.* two of the points shown in the previous figures. Then, considering these two points only, *and not any particular planes passing through them*, reflection is possible only when the path differences between the beams from A and from B differ by a whole number of wavelengths. For the first order reflection, AP, the geometrical relation required to fulfil this condition is

$$\lambda = b \sin \alpha_1, \qquad \ldots(3)$$

and for the second and third orders, AQ and AR, the corresponding relations are

$$2\lambda = b \sin \alpha_2, \quad 3\lambda = b \sin \alpha_3.$$

All first order reflections from planes passing through A and B must therefore lie along the cone APP', all second orders along AQQ' and so on. If the crystal is continually rotated around the line AB then these reflections will appear. It is to be noted, however, that since any particular plane will only reflect at its own glancing angle, θ, determined by the interplanar spacing, then each plane will reflect only four times per revolution, once upwards to the right, once upwards to the left, once downwards to the left, and once downwards to the right. This will be demonstrated again later. All four beams from each set of planes lie

along the surface of the cone. If, now, the reflected radiation is received on a photographic film formed into a cylinder with the rotating crystal axis as centre, then the reflections will lie along a series of straight lines at right angles to the rotation axis. If, on the other hand, a flat film is used, then the reflections lie on a series of hyperbolae, as in Plate II (Fig. 1), since the intersection of a cone with a plane is a hyperbola.

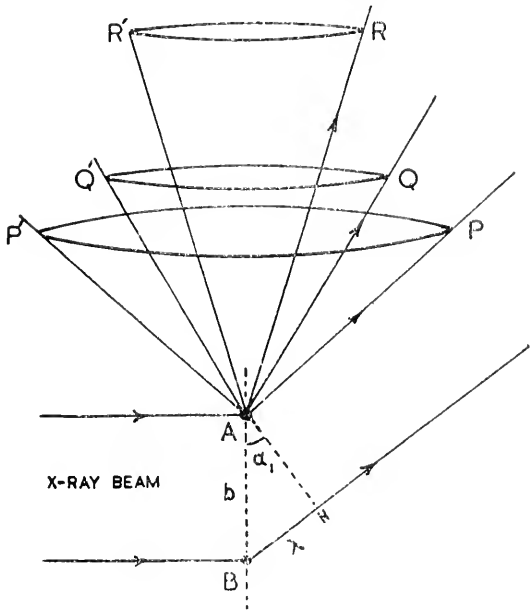

FIG. 17. *The rotation diagram.* For construction, see text. The radiations at *A* and *B* are "in step". Reflection will occur along *AP* if

$$BN = n\lambda,$$

where *AN* is perpendicular to *BN*, *i.e.* if

$$b \sin \alpha_1 = n\lambda,$$

where n is an integer.

For the direction *AP*, $n = 1$, so that

$$\sin \alpha_1 = \lambda/b.$$

The angle of the reflected ray to the line *AB* is therefore fixed. Its direction is otherwise not specified, so that reflection can occur anywhere from *A* along the conical surface *APP'*. Similarly for *AQ* ($n=2$) and *AR* ($n=3$). The condition $n=0$ corresponds to the horizontal plane containing the primary beam.

These hyperbolae are called the *layer lines*. This latter procedure is the one usually adopted with fibres. There is now an easy method for calculation of the primitive translation *b*. Thus, if *r* is the distance on the plate between the apex of the hyperbola (or the line in cylindrical films) and the centre of the photograph (or, better, one-half the distance

between the apices of two corresponding hyperbolae, one in the upper and one in the lower half of the diagram) then clearly

$$r/D = \tan \alpha_1,$$

hence α_1 can be calculated and b determined from equation (3).

For reasons into which we need not go, but will be obvious after a little thought, there is never a reflection at the apex itself; hence the calculation is usually made from other spots on the hyperbolae. This complicates the mathematics, but the principle remains the same.

Now this process applies strictly also to fibrous cells. If a bundle of cells, say of ramie fibres, is prepared in such a way that the fibres lie strictly parallel to each other and this bundle is mounted in the X-ray beam as in Plate II (Fig. 1), then the "fibre diagram" obtained is essentially a rotation diagram, as will be clear from the figure. The reason for this is obvious. In the first place, this is a *bundle* of fibres in which the fibres lie parallel but otherwise in random orientation about their lengths. Hence the photograph will naturally be that of a single fibre rotated about its length. More than this, however, each single fibre is a hollow thread with an axis of symmetry parallel to its length. Hence the diagram of a single fibre corresponds to that of any part of its wall rotated about the cell axis. The pattern of spots in the diagram makes it clear that one axis of the unit cell lies almost parallel to the fibre length and this makes calculation of one side of the unit cell a very simple matter.

The unit cell of cellulose

The parameter b for cellulose turns out to be 10·3 Å., or very nearly that, for every type of cellulose examined. This is formally regarded as the b axis. It will be noticed that a reflection corresponding to a spacing of 10·3 Å. does not actually appear in the diagrams of cellulose. This must mean that, between planes passing through neighbouring lattice points and parallel to the *ac* plane there must be at least one other plane interleaved, spaced $b/2$ Å. apart (for then reflections from successive planes spaced b Å. apart with a path difference of 1λ will be obliterated since the path difference for the interleaved planes will be $\lambda/2$. As a matter of fact the first strong reflection to appear is often the fourth order, indexed therefore 040, and corresponding to an interplanar spacing of 2·56 Å. (approximately 10·3 Å).

Determination of the other two axes of the unit cell is not so simple since, in view of the cylindrical nature of the cells, it is impossible to obtain rotation diagrams about axes other than the b axis. Recourse

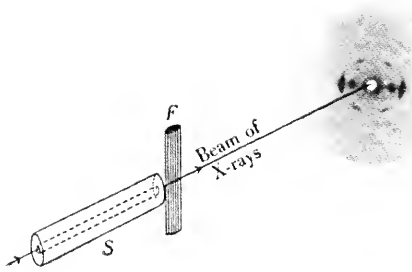

FIG. 1. Diagrammatic representation of the method used in obtaining X-ray diagrams of fibrous material. The beam is collimated by a cylindrical slit, *s*, 0·5 mm. diameter, made of some material impervious to X-rays, such as lead. The fibre bundle *F* is placed up against one end of the slit and perpendicular to the beam. The diffracted rays are received on a photographic plate and are recorded there as arcs. The diagram shown here is that of ramie fibres. In all subsequent X-ray diagrams in this book the cell length lies, as here, parallel to the longer edge of the page.

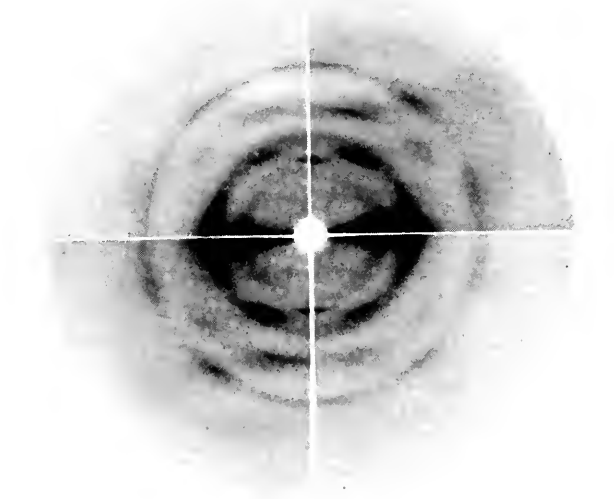

FIG. 3. X-ray diagram of hemp fibres before and after delignification. A mask of brass was placed over the photographic film in such a way that only two opposite quadrants in a circular aperture were exposed at one time. The two specimens (treated and untreated) were therefore photographed on one film and therefore processed together. This reduces to zero any complications due to variation in development technique, etc.

Upper right and lower left: Untreated hemp fibres.
Upper left and lower right: Fibres after lignin extraction.

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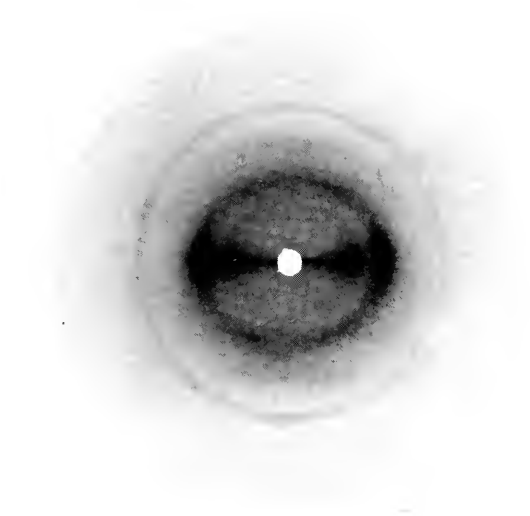

FIG. 2. (*a*) X-ray diagram of wood from the 20th annual ring in a stem of *Picca abies*. Beam perpendicular to grain of wood. CuK_α radiation.

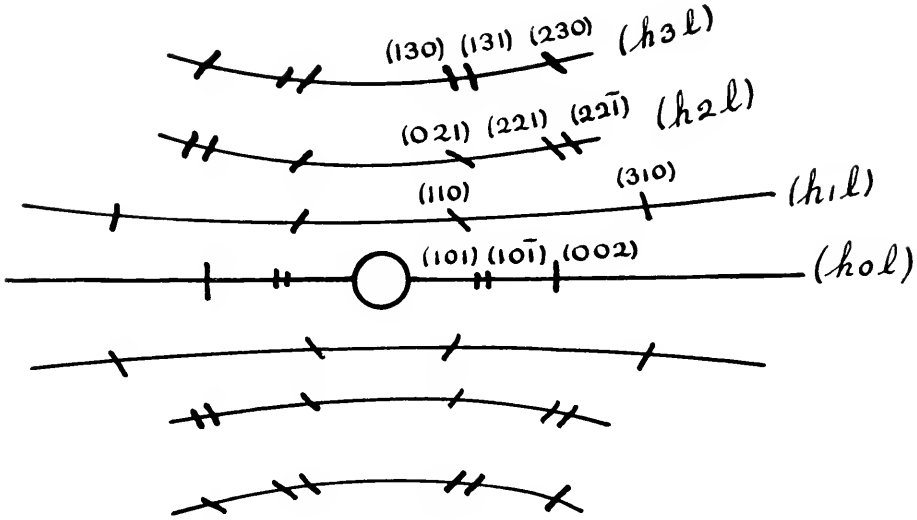

FIG. 2. (*b*) Chart corresponding to fig. 2 (*a*), showing the indices of the planes which give rise to the various arcs.

must therefore be made to more indirect methods. These involve a trial and error method; in principle, reasonable values are assumed for a, c and β, followed by a calculation, from the unit cell thus determined, of the spacings d and the indices hkl for all possible planes, and a comparison of these with the spots on the diagram. The method is nowadays rendered much more workable by the use of the concept of a reciprocal lattice which enables the spots on a rotation diagram to be indexed

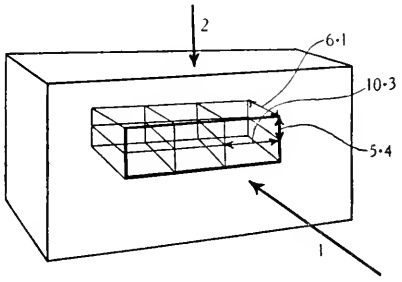

FIG. 18. Diagrammatic representation of the investigation of a plate of cellulose as it occurs in, for instance, the tunicates. Six unit cells are drawn (cp. Fig. 19) with the b axis horizontal and the *spacings* 10·3, 6·1, 5·4 Å. are indicated. When an X-ray beam is directed along 1, reflections are observed from planes spaced 5·4 Å. apart (since there is in a natural specimen some angular dispersion around the axis) but not from the 6·1 planes (since these stand at right angles to the beam).
When the beam lies along the direction 2, then reflection is observed from the 6·1 Å. planes only. Hence by rotating the specimen about the b axis, the angle of rotation between the positions of maximum reflection from 5·4 Å. planes and from 6·1 Å. planes, after a correction for the (small) difference in glancing angle, gives a rough measure of the angle β.

(see *e.g.* 13). In order to choose reasonable values for a and c, it should be noted that along the horizontal line of the diagram of ramie fibres (Plate II, Fig. 2 (*a*)) there are three principal arcs on each side of the centre; these, counting from the outer arc to the inner, correspond to planes spaced 3·9, 5·4, 6·1 Å. apart and lying parallel to the b axis. This makes it reasonable to suppose that the unit cell is at least monoclinic and, as a first trial, any two of these three spacings could be equated to the two unknown sides of the unit cell. The first suggestion, made by Sponsler (14), was that $a=6{\cdot}1$, $c=5{\cdot}4$ when it was found that if $\beta=88°$ then

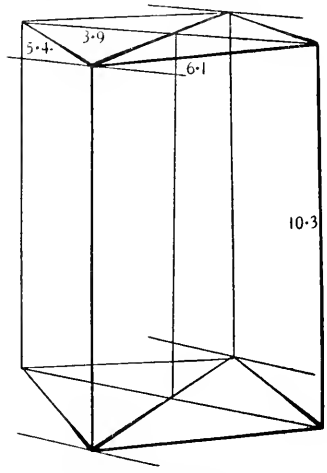

FIG. 19. The unit cell proposed by Sponsler.

most of the arcs could be accounted for. This would make, for instance, the planes spaced 3·9 Å. apart the 101 planes (Fig. 19). Verification that the angle β is of the order of 90° was soon forthcoming in the observations made on *plates* of cellulose such as occur in tunicates and in the green alga *Valonia* (see Fig. 18). The unit cell of cellulose could then be imagined to be as in Fig. 19. This was soon followed by a new suggestion (15) in which the axes were taken as:

$$a=8{\cdot}34 \text{ Å.} \qquad c=7{\cdot}9 \text{ Å.} \qquad \beta=84°$$

a scheme in which the planes of 6·1 Å. spacing become the 101 planes, those of 5·4 Å. spacing the $10\bar{1}$ planes and the 3·9 Å. planes the 002. Geometrically this scheme is equivalent to that of Sponsler (Fig. 20) and, it should be noted, demands a point in the middle of each unit cell identical with those at the corners. This latter scheme of Meyer and Mark's was accepted with little question until comparatively recently (see 16) and will be adopted here since possible modifications are of no moment for the discussion which follows. Space will not allow any attempt to show how the various diffraction arcs in the cellulose diagram can be indexed from either of the two unit cells proposed, nor is it the purpose of this book to make any such attempt. For the sake of completeness, and for future reference, however, the indices of some of the reflections are given in Plate II, Fig. 2(*b*), and it may perhaps be noted that some of the indices can, in point of fact, be allocated by inspection. Clearly, since all reflections on the equator arise from planes parallel to the *b* axis, the index *k* must uniformly be zero, *i.e.* all equatorial reflections have the indices *h*0*l*. On the first layer line, where the path difference between neighbouring lattice points is one wavelength, the index *k* must be 1 (see p. 40) so that all reflections on this layer line must be *h*1*l*. Similarly all reflections on the second layer line must be *h*2*l*, on the third *h*3*l* and so on.

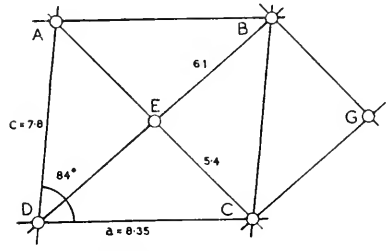

FIG. 20. Part of the *ac* plane in cellulose. *EBGC* represents the unit cell of Sponsler. *ABCD* represents the unit cell proposed by Meyer and Mark. The two are clearly identical in a geometrical sense.

The arrangement of glucose residues within the unit cell

The final step which remains to be taken is to place the atoms of which cellulose is composed in their proper places within the unit cell. With

simpler crystals this can often be achieved by calculation from the intensities of the various diffraction spots. Unfortunately cellulose is far too complicated a substance for the usual crystallographic techniques to be used here; the number of atoms in the unit cell is too large and the number of diffraction arcs is too small for these calculations, apart from the fact stressed earlier that the substance can be investigated only in the form provided by nature. Hence recourse must be had to

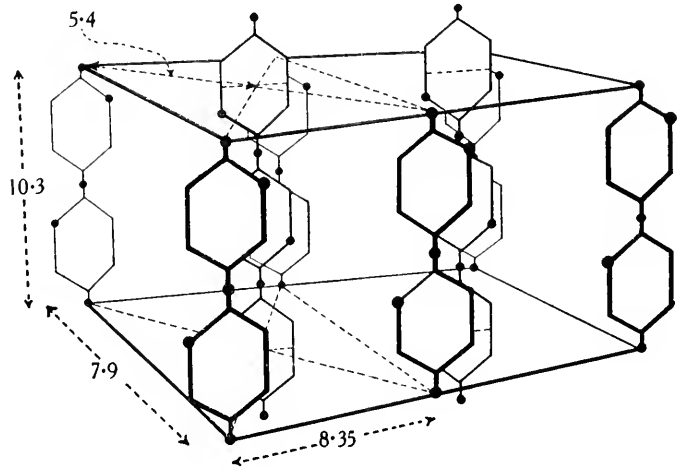

FIG. 21. Two unit cells of cellulose, as suggested by Meyer and Mark, with the cellobiose residues in position. Note that the chain lying along the central line of each unit cell is oriented in the opposite sense to those at the corners. The reasons for this cannot be discussed here, but it should be mentioned that the evidence for this regular alternation is unconvincing.

indirect methods. The significant fact to be noted is that the length of the *b* axis, 10·3 Å., is exactly the length of the cellobiose molecule suggested by Haworth, and it seems therefore very reasonable to orient the cellobiose residues in the unit cell as in Fig. 21, by placing one such residue along one side of the unit cell and remembering that by definition all four sides, and the central line, are identical. Further, by remembering that the whole structure of the space lattice of cellulose can be built up by superposition of such unit cells, it may be seen that the cellobiose residues are joined up end to end into long molecular chains as in Fig. 22. The conception is thus immediately reached of long molecular chains of cellobiose units as pre-existing in cellulose. Further, the mere existence of an X-ray diagram thus implies that in at least some regions in the cellulose structure the chains lie strictly parallel to each other and spaced regularly the same distance apart. That this

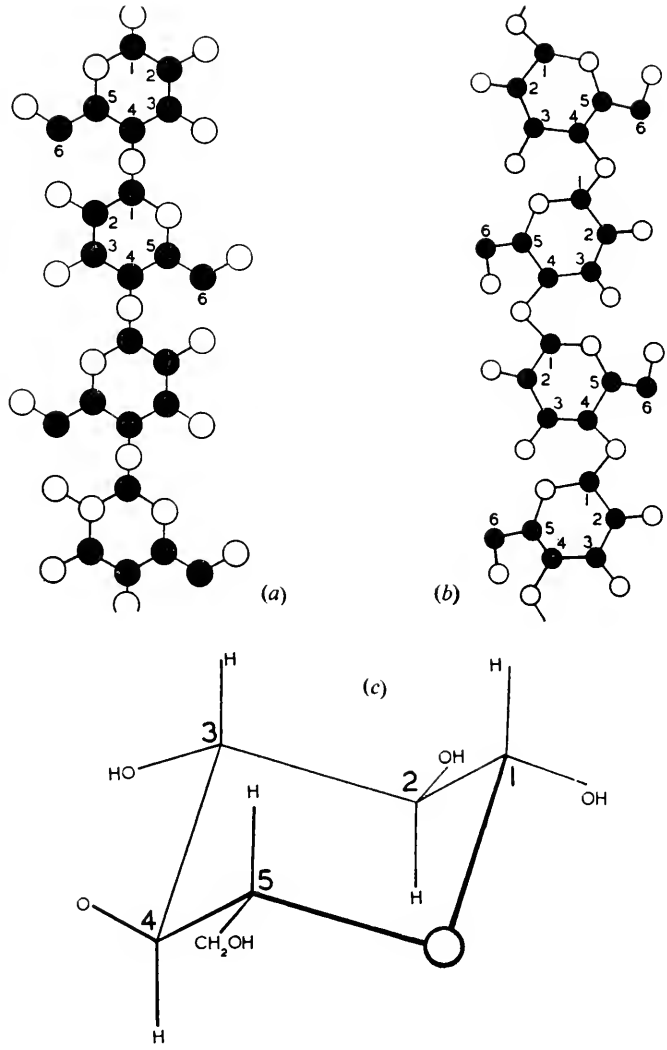

FIG. 22. Parts of a molecular chain of cellulose. Hydrogen atoms are omitted.

(*a*) Diagrammatic two-dimensional representation of 4 β-glucose residues united in a chain. This represents the organization of a molecular chain as first proposed by Meyer and Mark, and corresponds to fig. 21.

(*b*) Diagrammatic two-dimensional representation of 4 β-glucose residues in a chain following the suggestions of Stuart. The consecutive glucose residues lie in a zigzag about the 1:4 C—O—C link. This kind of folding allows the cellobiose residues to fit into the unit cell 10·3 Å. long without the strain imposed on the C—O—C bond necessary in the Meyer and Mark model.

(*c*) In figs. 22(*a*) and (*b*) the rings of glucose residues are of necessity drawn flat in the plane of the page. This figure is intended to convey some idea of the spatial relationships of the ring. The ring oxygen (open circle) and carbon 5 lie in the front of the drawing and carbons 2 and 3 behind. Carbons 1 and 4 can be regarded as lying in the plane of the page.

regular arrangement is not, in fact, uniformly a feature of the structure is also evident from the diagram. It will be noted that in the diagrams shown in Plate II, Figs. 1 and 2, the diffraction arcs lying along the meridian are rather narrow radially, while along the equator all the arcs are very broad radially. The beams of X-rays diffracted from

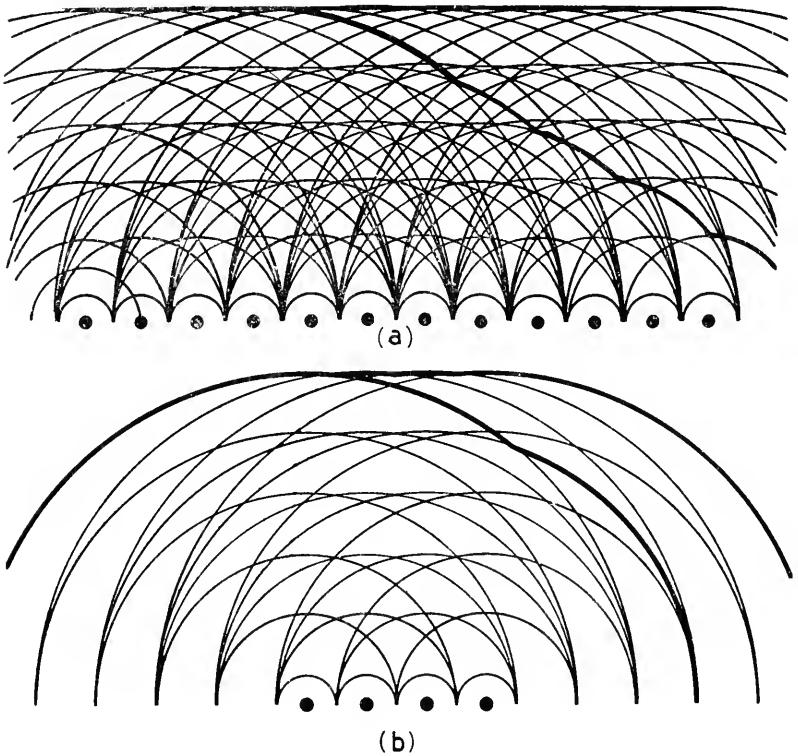

FIG. 23. Diagrams to illustrate the effect of the number of diffracting points on the character of the diffracted beams. In both figures the radiation is conceived as travelling parallel to the surface of the paper, from below upwards. The diffracting points are indicated by black circles, and the waves diffracted from each such point as circles drawn with these points as centres. (*a*) Many diffraction points. The constituent, circular wave fronts result in the transmission of the incident beam in the original direction. At the same time a wave front is reflected at a considerable angle to the incident wave front. Both are emphasized in the diagram by thickened lines (the phenomenon is best observed by holding the page on the level of the eyes and glancing along the horizontal thickened line). If the page is now rotated about a vertical line, it will be observed that tangents to a series of circles become collinear when glancing along the sloping thickened line. Both wave fronts are sensibly straight and the records on a photographic plate would be clearly defined spots. (*b*) Few diffraction points. Two wave fronts are delineated as in (*a*). Here, however, both have curved margins. The record on a photographic plate would therefore be two diffused images, rather denser towards the centre and gradually decreasing in density towards the outside.

planes lying parallel to the molecular chains of cellulose are therefore much broader than are those from planes perpendicular to this direction. Explanations of this broadening are well recognized (Fig. 23); it implies that in any crystalline region of the cellulose the number of planes lying parallel to the chains is less than that of the planes lying perpendicular to them so that, in the sense that the regions of strictly regular arrangement of the chains are limited in extent, we may speak of the existence in cellulose of "crystallites" or, to use the older term "micelles". It should, however, carefully be noted that this evidence from the X-ray diagram

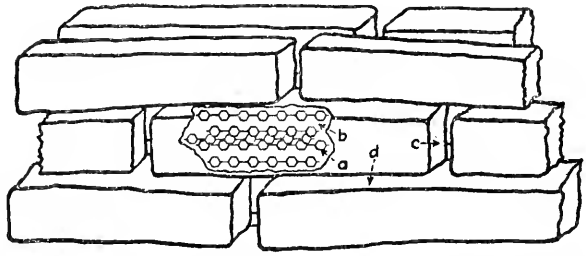

FIG. 24. The micellar structure of cellulose originally propounded. (Reproduced from *Protoplasm*, by W. Seifriz, McGraw-Hill, 1936, by courtesy of the author.)

does not necessarily imply the existence of discrete micelles, in the sense originally used by Nägeli(17). All that it says is that there are periodic interruptions in the lattice, and it is now generally agreed, on evidence of many other kinds some of which will be discussed later, that the original hypothesis of the existence of distinct and separate micelles as first postulated by Nägeli (Fig. 24) must be modified in such a way that the distinction between "micelle" and "intermicellar space" is merely as between ordered and less ordered cellulose chains (Fig. 25). This newer hypothesis has the obvious advantage that one no longer needs hypothetical long-range forces to hold the micelles together and it also yields a better explanation of the tensile properties of fibres (see 3, 29). It has been calculated (22) that the "micelles" are about 50 Å. in diameter and at least 600 Å. long. It will be seen later that the molecular chains of cellulose themselves are far longer than 600 Å.

Perhaps, before leaving the principles of the X-ray method, one further point may be made, which will come under discussion again later on. Adopting the dimensions for the unit cell proposed by Meyer and Mark (or the one by Sponsler, for the result will clearly be the same) the density of the cellulose within the crystalline regions can be determined. This is done by noting that each unit cell has two cellobiose residues in it (the one at each edge being shared between four unit cells).

We therefore merely calculate the weight of these residues and divide by the volume of the unit cell. Thus:

$$d=(2)(324)(1{\cdot}6604)/(10{\cdot}3)(8{\cdot}35)(7{\cdot}9)\sin 84^\circ=1{\cdot}59.$$

The density of cellulose never in fact reaches this figure and this is undoubtedly due to the presence of periodic breaks in the regularity of structure as mentioned above. It should be clear that the density of

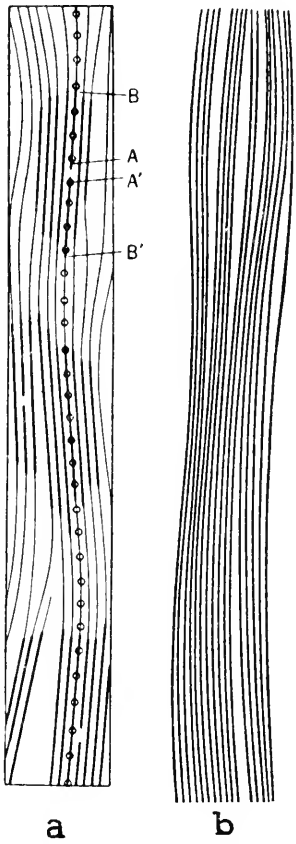

FIG. 25. Modern conceptions of micellar structures.

(*a*) The "fringed micelle" as suggested by Kratky and Frey-Wyssling.

(*b*) The continuous, deformed structure proposed by Meyer (1940). (Reproduced from *High Polymers*, Vol. IV, *Natural and Synthetic High Polymers*, by K. H. Meyer, Interscience publishers, 1942 (29(*b*)), by courtesy of the author.) In the particular interpretation given in (*a*) two molecular chains such as those delineated by circles behave as a single chain traversing the whole structure since the ends *A*, *A'*, lie in the crystalline region (marked by dark lines).

real fibres will depend not only on the kinds of incrusting substances present but, even after these have been removed, on the relative proportion of the "crystalline" to the "non-crystalline" material. For obviously the lack of regularity in the packing of the chains in non-crystalline regions can lead only to a decrease in density. This effect in turn may well depend on the degree of orientation of the micelles, and so it is understandable that the density may vary widely and in a manner dependent in quite a complicated way upon structure. Density determinations do, however, yield a rough idea of the ratio of crystalline to non-crystalline cellulose, a figure as low as 1·53 for instance, implying almost 100% non-crystalline material, and a figure of 1·59 or 1·60 purely crystalline. There are, naturally, many other ways of determining the crystalline/non-crystalline ratio, both physical(18) and chemical (19) but these will not be discussed here. Just as, however, in technology the amount of non-crystalline material present in a fibre is of paramount importance to the properties of the fibre, so also here the amount of non-crystalline material may be of very considerable importance and it will be found necessary to refer to it again and again in the following pages.

Analysis of structure by optical methods

The method of X-ray analysis thus briefly described has, unfortunately, certain rather severe limitations as applied to botanical objects. As normally used it is necessary to work with material a few millimetres long and about one millimetre thick in the direction of the X-ray beam, and therefore with a whole tissue. Since even the simplest tissue can be composed of more than one cell type, and since the wall structure of each single cell may differ from point to point in the wall, it is sometimes a matter of difficulty to give a correct interpretation in terms of *single* cell walls—with which the botanist is most concerned—and the way is open dangerously wide for serious misinterpretations. These have, in fact, occurred in the literature. Certainly there is no theoretical reason why spectrometers should not be designed to work with the smallest pieces of plant cell wall which could be handled, but in practice there are limits below which it is not at the moment feasible to go. For instance, using the normal sealed-off types of X-ray tube and with a specimen-object distance of 3 cm., then a piece of wood as thick as a match-stick will give a reasonably intense diagram in about two hours. If one needs to investigate, however, a single cell, then the exposure time is increased enormously. Firstly, the diameter of the slit through which the X-rays impinge on the specimen must be reduced from the customary

0·5 mm. to a size comparable with that of the specimen. This is necessary, not only for the need to mount the specimen in the beam with no foreign body also in the beam, but also because however carefully the slit is designed, there is always some scatter from the edges of the slit and this must be kept well below the scatter from the specimen. This alone, therefore, may increase exposure time by a factor of 10× or more when working with single cells. Again, the amount of reflecting material is reduced perhaps by a factor of 800×. This enormous increase in exposure time can to some extent be offset by reducing the specimen-film distance say to 2 mm., which would decrease the exposure necessary by a factor of 900/4 (since beam intensity varies inversely as the square of the distance) and by other methods, but nevertheless the size of the specimen cannot be reduced indefinitely without a very serious increase in exposure time. In point of fact, however, X-ray diagrams have been published of single cells (20) and of even smaller botanical specimens (21), even under conditions in which the exposure time necessary was of the order of 150 hours. For some of the details of structure on plant cell walls which it is very necessary to investigate, however, it is as yet impossible to employ an X-ray technique and recourse must then be made to optical methods. It is largely for this reason that the polarizing microscope is of such importance in botanical investigations. At the same time it must be stressed that this is by no means the only reason why this instrument provides such a useful tool; it can and does give information which could be obtained in no other way.

Polarized light and structural asymmetry

The interaction of matter and light is usually expressed by the *refractive index*, which is commonly interpreted in terms of a bending of the rays of light when passing obliquely from one medium to another. If the rays impinge, for instance, on the surface of separation of a block of glass in air at an angle i to the normal, and the refracted rays make an angle, in the glass, of r to this normal, then the refractive index is expressed as $\sin i/\sin r$. It is more instructive, and more apposite to the needs of the following discussion, to define refractive index in another way. When light passes from a less dense medium (say air) to a more dense (say glass) then its velocity is diminished, and the ratio of the velocities (air/glass) is numerically equal to the refractive index of the glass. As already noted (p. 36), such a ray of light consists essentially of a series of vibrations at right angles to the direction of propagation and, following the electromagnetic theory of light, it is known that these

vibrations correspond to an electric and a magnetic vector, each at right angles to the direction of propagation and mutually perpendicular, varying in time and space according to a sine law. The "direction of vibration" of the light corresponds to the direction of the electric vector and it follows, therefore, that light is affected by passage through a transparent body largely in terms of the electrical state of the matter of which the body is composed.

To understand this most clearly it is well to consider the theory which was first put forward by Bragg (23) to explain quantitatively the refractive indices of certain crystals. When light falls on an atom, the electrons in the atom suffer a displacement in view of the electric component of the vibration. The atom is said to be *polarized*,* since the positive nucleus and the cloud of negative electrons suffer a mutual displacement. When two atoms lie close together, as they do when they are linked chemically, then the polarization of one atom has an effect on the polarization of the other. This means that the interaction of matter and light depends in the first instance on the type of chemical bonding between the atoms of which the matter is composed. The mutual effect of one atom on another falls off very rapidly with distance and therefore in molecules, and in crystals composed of molecules, the total polarization is almost entirely confined to the mutual effects of chemically linked atoms. Now the refractive index n varies with polarization σ according to the relation $(n^2-1)/(n^2+2)=K\sigma$, where K is a constant; hence the higher the polarization the greater the refractive index. With rays of light, therefore, taken straight from an incandescent lamp for instance, the total effect of the passage through a crystal will arise as a sort of average effect of all the various types of bonding in the crystal structure. This is because in such rays of light the vibration (electric vector) lies, not in a single plane, but in any and all directions perpendicular to the direction of propagation.

Suppose, now, that the light is first passed through a Nicol prism, or one of its successors or through a sheet of Polaroid. Then the light issuing from any of these bodies is vibrating in one plane only; it is said to be *plane polarized.* Now the immediate phenomena associated with the passage of such a ray of light through a crystal is obviously more complicated; it will depend not only on the direction of propagation but also, for any one direction of propagation, on the direction of vibration. Consider, for instance, the case of a diatomic molecule

* The word *polarized* is unfortunately used in two senses. *Polarization* of atoms refers to the displacement of positive and negative parts. *Polarized* as applied to light refers to something quite different; this will be referred to later.

(Fig. 26). When the light is vibrating parallel to the line joining the two atoms (Fig. 26(*a*)) then the polarizing effect of the light on one atom is enhanced by the induction of the other (polarized) atom: dipole *A* increases the polarization of dipole *B* and dipole *B* enhances the polarization of dipole *A*. The polarization is therefore high. When, however, the molecule is turned through 90° (Fig. 26(*b*)) each dipole opposes the polarization of the other and the polarization is lower. In the first position, therefore, the refractive index is high and in the second

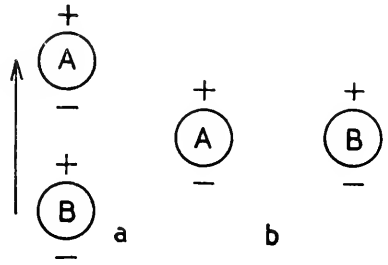

FIG. 26. Structural asymmetry in a diatomic molecule and the refractive indices. The direction of vibration of the light is parallel to the arrow.

(*a*) When the line joining the atoms is parallel to the direction of vibration, the polarization of each atom increases that of the other (since "unlike poles attract").

(*b*) When this line is at right angles to the direction of vibration the polarization of each atom reduces that of the other.

one lower. If a crystal could be imagined composed of such diatomic molecules, arranged strictly parallel to each other, *i.e.* in crystal form, then the direction of the line joining them could be determined by a study of the refractive indices of the crystal. In a fibre in which the chain molecules lie parallel to each other and to the length of the fibre, it would therefore be expected that the refractive index for light vibrating parallel to the length of the fibre would be very much greater than for light vibrating at right angles to fibre length; and this expectation is fulfilled since the refractive index of such fibres parallel to their length is of the order of 1·60 (n_γ) and at right angles to it 1·53 (n_α). Irregularities of structure and other effects can modify the refractive indices considerably, but these will be considered later as occasion arises: it should be noted here, however, that such irregularities will have the effect of reducing the value of n_γ and increasing the value of n_α. By observing the values of these two refractive indices, and their direction in the cell wall, a good deal of information concerning structure can be derived. Under certain circumstances, the direction of the cellulose

chains can be determined unequivocally in even the smallest microscopically visible piece of wall, and the actual value of the refractive indices gives some information (commonly rather difficult to interpret, however) concerning irregularities. Investigation of wall structure by optical methods involves, therefore, directly or indirectly, the determination of refractive indices. For many purposes it is, as a matter of fact, unnecessary actually to determine the value of the indices but sometimes the information to be sought can be obtained only by careful measurement. The method involved may therefore briefly be described before passing to the commoner methods employed.

The measurement of refractive indices

In principle the method used for objects of microscopical size is a very simple one. It involves nothing more than finding a liquid medium in which there is no bending of the rays of light at the edge of the fibre, so that the fibre becomes almost invisible. It never does in practice become completely invisible for a number of reasons into which we need not go at the moment. Since the refractive index varies with the wavelength of the light used, it is customary to use monochromatic radiation given by a sodium vapour lamp, *i.e.* to use the sodium D lines. The method can be tedious, but if a set of liquids is prepared beforehand differing in refractive index by, say, 0·01, and a sufficient quantity of material in an adequate condition is at hand, then results come more quickly than might be imagined.

As a guide to the choice of the correct liquid a phenomenon first described by Becke and called the Becke line is employed. If the boundary between two media is observed under a microscope then a line of light is visible along the boundary if the refractive indices of the media are different. On raising the microscope objective, this line of light moves into the medium of higher refractive index. It is, therefore, a matter of a moment to decide whether the immersion liquid bathing a fibre has an index lower or higher than that of the fibre and thus to obtain a choice for the next liquid to be tried. The method, then, is briefly this. The fibre is mounted in a suitable medium of known refractive index on the rotating stage of a polarizing microscope. Below the condenser of the microscope is a Nicol prism, or some corresponding polarizing device, and this is left in position. A second Nicol, either in the body tube or the eyepiece depending on the design of the microscope, is thrown out of the optical system, after using the combination of the two prisms, by a method to be described later, to set the fibre with the direction of, say, the higher refractive index parallel to the direction

of vibration of the light issuing from the polarizer. Observation of the Becke line will then show whether the refractive index of the medium is too high or too low. If it is too high, for instance, then a liquid of a lower index can be tried until the refractive index of the fibre is "bracketed"; when by progressively narrowing the upper and lower limits, an increasingly close approximation to the refractive index of the fibre can be made. With care and under appropriate conditions, the refractive index can thus be determined to the third place of decimals. To obtain the necessary gradation in refractive indices it is necessary to use a mixture of liquids and a number of liquids are available (12, 24). A very useful combination is *a*-monobromnaphthalene and liquid paraffin which fulfils the requirement that neither component shall swell the fibre (see below) and each shall have about equal volatility so that the refractive index of any mixture shall not change appreciably during the observation.

The interpretation of refractive indices

Although, however, the technique of refractive index determination does not present any great difficulties, the precise interpretation is not always very easy. This arises in virtue of the heterogeneity which has been shown to be a feature of the organization of cellulose (Fig. 25). Since cellulose is composed partly of chains arranged quite regularly in crystalline fashion and partly of chains with a varying degree of randomness, then the refractive indices of the "micelles" will differ from those of the non-crystalline material between, and therefore from the refractive indices of the fibre as a whole. Most often, and particularly in botanical research, it is the refractive indices of the micelles which would seem to be wanted, since these give some idea of the regularity of arrangement of the micelles with respect to each other; and so long as the older ideas of discrete micelles remained feasible (Fig. 24) then it seemed possible, by choosing non-swelling media which would nevertheless penetrate the intermicellar spaces, actually to determine micellar refractive indices. Now we realize that this was an over-simplification then it is obviously practicable only to measure the refractive indices of the fibre as a whole, and this is the procedure always adopted. It is then possible under certain feasible assumptions, to calculate the micellar refractive indices, *i.e.* the indices which the fibre would have if it were composed entirely of cellulose chains arranged in crystalline fashion.

Theoretically there is another effect which must be taken into account, an effect which bulked largely in the development of the micellar hypothesis of cellulose structure but which is, however, of little practical

importance in fibres. This is the Wiener effect. If a number of isotropic rods of refractive index n_1 are immersed in a medium of different refractive index n_2 then, provided that the diameter of the rods is small compared with the wavelength of light, the system has refractive indices which are different for light vibrating parallel to the rods and perpendicular to them. In other words the system is behaving like a crystal, and, more particularly, the refractive indices of the whole body depend both on that of the rod and that of the medium. They therefore change as the medium is changed. Fortunately this effect seems to be of little importance in cellulose, largely no doubt because the intermicellar spaces are in fact filled with cellulose chains, and the refractive index of a mixture of random cellulose chains and any of the media commonly employed is never very different from that of dry cellulose itself.

Nevertheless it is clear that the refractive indices of the fibre as a whole may be considerably different from that of the micelles, particularly if large amounts of incrusting material are present. It is well, therefore, that the ordinary mixture formula, as used for liquids, holds to a sufficiently close degree of approximation (in the sense of giving consistent results). Thus if the refractive index of a dry fibre parallel to its length is n_γ, that of the crystalline portion $n_{\gamma cr}$, of the non-crystalline $n_{\gamma a}$ and of other substances n_i, then the following relation may be set up

$$n_\gamma = fn_{\gamma cr} + an_{\gamma a} + in.$$

where f, a, i are the relative volumes ot the crystalline cellulose, non-crystalline cellulose and amorphous substances respectively and $f+a+i=1$. Similarly, for the refractive index for light vibrating perpendicular to fibre length,

$$n_\alpha = fn_{\alpha cr} + an_{\alpha a} + in_i.$$

Hence, provided the assumption is made that the non-crystalline cellulose is amorphous in the optical sense (*i.e.* that its refractive index is invariate with vibration direction), then the difference between the two refractive indices of a fibre is roughly a linear function of the amount of crystalline cellulose present. It is clear from these considerations that the refractive indices of a fibre will depend on its water content, so that this must be standardized very carefully indeed in comparative estimations. This is usually done by drying the fibre over phosphorus pentoxide (24, 25).

The index ellipsoid

Thus far reference has been made to only *two* refractive indices for a fibre through which light is passed in a direction perpendicular to its

length, and it has been seen that the refractive indices depend on the direction of vibration of the electric vector. In fact, there *are* only two refractive indices for light passing in this direction; the light vibration is resolved in the fibre into two vibrations, one parallel and one perpendicular to the length of the cellulose chains, with vibrations in no other directions. This will be illustrated experimentally later on. The question now arises, however, as to the effect on the refractive indices of a change in the direction of propagation of light through the fibre. This is a very practical problem, for we are very unlikely always to meet cells so beautifully organized that their cellulose chains lie strictly parallel to the fibre length, as we have supposed up to now in the theoretical examples considered. In addition, in any case, we often do need to observe fibres in more than one direction. It becomes therefore essential to outline the variation of refractive indices with direction of propagation.

It has been seen that the refractive index for light vibrating along the length of the cellulose chain is much greater than in any direction perpendicular to it, and that this is understandable in terms of the structure of cellulose. Similarly it should be noted that since the molecular chains of cellulose consist of a series of rings which, although somewhat puckered, are nevertheless rather flat, then for light PROPAGATED *parallel* to the length of the chains, the refractive index for light VIBRATING *parallel* to the planes of the rings should be different from that perpendicular to this plane. In other words, cellulose should have a large, a medium and a small refractive index. So far as the writer is aware, although there are vague statements in the literature that this is actually true, there is no real evidence for the existence in cellulose of more than two refractive indices in this sense; in fact it is difficult to see how such evidence could be obtained. Cellulose can therefore be considered, and is usually considered, to have only two principal refractive indices. This is one example of the class of crystals called *uniaxial.*

If, in such crystals, the refractive indices are measured for some direction of propagation other than those so far considered, then it is found that all these refractive indices can be correlated in the following way. Suppose an ellipsoid is constructed (Fig. 27) with half the major axis numerically equal to n_γ, the largest refractive index, and lying parallel to the direction of the cellulose chains, and with half the minor axis numerically equal to n_α, the smallest refractive index. Then, remembering that the refractive index depends on the *direction of vibration* of the light, the refractive indices for a ray of light running in

any direction OX are given by the major axis OA and the minor axis OB of the elliptical section of the ellipsoid made by a plane normal to the direction of propagation and passing through O. It is obviously merely a matter of geometry to calculate, from known values of n

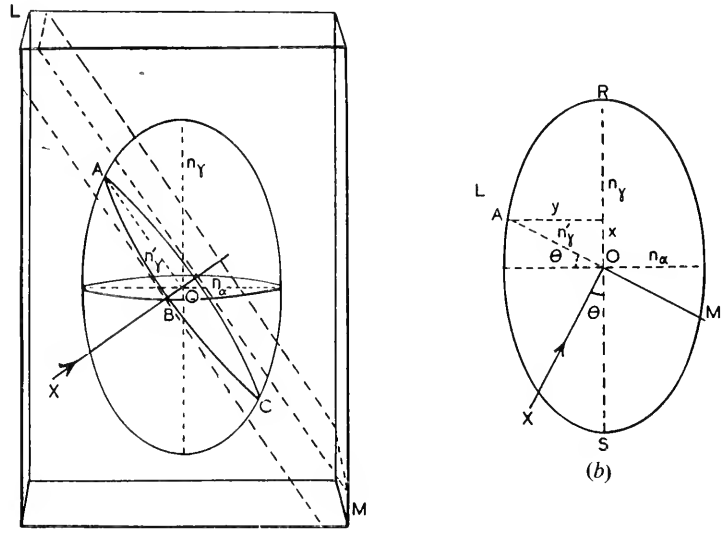

FIG. 27

(*a*) Diagrammatic representation of a piece of wall cut with sides parallel to the chain direction and the index ellipsoid drawn in. The ray of light OX is supposed to be propagated from below upwards towards the front. The ellipse ABC is the section of the ellipsoid made by a plane normal to OX. The corresponding refractive indices are OA and OB. Note that OB is always equal to n_α.

(*b*) If a section such as LM (Fig. 27(*a*)) is cut and examined by light propagated in the direction OX, *i.e.* normal to the cut surface, then the refractive indices can be calculated as illustrated here. ARS is the section of the ellipsoid containing the direction of propagation OX and the major axis of the ellipsoid. OA is at right angles to OX and therefore represents the refractive index required. From the equation of the ellipse, if A is the point (x, y)

$$\frac{x^2}{n_\gamma^2}+\frac{y^2}{n_\alpha^2}=1,$$

i.e.

$$\frac{(n_\gamma')^2\sin^2\theta}{n_\gamma^2}+\frac{(n_\gamma')^2\cos^2\theta}{n_\alpha^2}=1,$$

$$(n_\gamma')^2=\frac{n_\gamma^2 n_\alpha^2}{n_\gamma^2-(n_\gamma^2-n_\alpha^2)\sin^2\theta}.$$

and n_α, what the refractive indices will be for light passing in any direction. The ellipsoid is known as the *index ellipsoid.* In any section of wall material, therefore, say the section LM, Fig. 27(*a*), light passing through the section at right angles to its surface will be split up into

two components, one vibrating parallel to *OA* and one to *OB*, and with the corresponding refractive indices as in Fig. 27(*b*), and these refractive indices can be calculated if n_γ, n_α and θ are known. Conversely, if the refractive indices for sections making known angles to each other are known (actually only two sections are necessary) then n_γ and n_α can be calculated as well as their orientation with respect to either section.

The use of crossed Nicols. The major extinction position

A good deal of useful information can, however, be derived without actual measurement of refractive indices. The principles involved can perhaps best be seen in botanical material by considering the wall of a fibrous cell in longitudinal section, *i.e.* the wall *BC* in Fig. 28. Suppose a polarizing microscope is set up, in the usual way, with the direction of vibration of light in the lower Nicol prism (the polarizer, below the substage) and the upper one (the analyser, above the objective) at right angles to each other. Then the light coming up from the polarizer, vibrating exactly at right angles to that which the analyser will allow to pass, fails to penetrate to the eye-lens, and the microscope field is dark. If now the cell wall section we are considering is placed in the field of view then, in general, it will appear bright against the dark background. If the stage is rotated it will be found that the wall will darken completely four times per revolution. These four positions are exactly at right angles to each other; in two of them the edge of the wall is parallel to the direction of vibration of the polarizer, and in the others that in the analyser. The explanation is as follows:

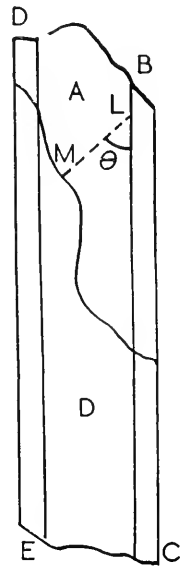

FIG. 28. Diagrammatic representation of part of an elongated cell. *BC*, *DE* are the thick side walls as seen in optical section. In region *A* the upper wall has been removed; in *E* both walls are present.

Looking down the microscope, let *PP* (Fig. 29(*a*)) be the direction of vibration in the polarizer and *AA* that in the analyser, and let the wall *BC* be inserted with one edge making any angle θ with the direction *PP*. Then the vibration along *PP* will have a component along both *BC* and *DE* (the n_γ and n_α directions). Two vibrations will then pass upwards through the specimen. Since, however, the refractive indices of these are different, their velocities are different (which is saying the same thing, see p. 51). Hence since the frequency, ν, of the light (the number of vibrations per second)

must remain constant, then the wavelength λ must be different since $\nu\lambda=c$, the velocity of light. Hence, as will be clear from Fig. 29(*b*), the two vibrations which pass into the wall in step, will emerge out of step and will not reconstitute the same vibration *PP* as went in. The light passing from the wall will therefore have a component along *AA* and the wall will appear bright. If, however, the specimen is turned so that the direction *BC* is parallel to *PP*, Fig. 29(*c*), then the vibration *PP*

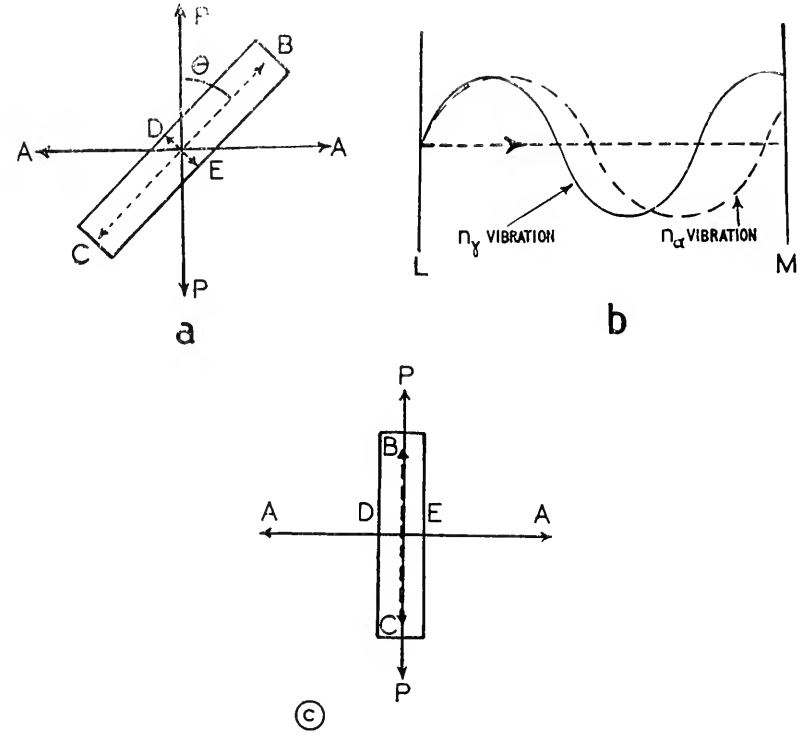

FIG. 29

(*a*) For explanation, see text.

(*b*) *LM* represents the wall thickness through which light is passing from left to right. For convenience in drawing, both vibrations in the wall are made to lie in the plane of the paper although they are actually at right angles to each other. The two vibrations enter the wall at *L* in step, but leave at *M* out of step.

(*c*) For explanation, see text.

obviously passes unchanged and is therefore extinguished by the analyser; the specimen is black. Similarly, when *PP* is made in turn parallel to *ED*, *CB* and *DE*. These positions of darkness are referred to as the extinction positions. Two of them, corresponding to the larger of the two refractive indices, correspond to the *major extinction position* (referred

to subsequently as the m.e.p.) and this in turn may be seen from Fig. 27 to correspond to the projection, in the plane of the section, of the direction of the cellulose chains in the wall. To determine this latter direction it remains therefore only to ascertain which of these two extinction positions is the m.e.p.

Newton's colour scale. The determination of the m.e.p.

This can be done quite readily after noticing that, if white light is used, the specimen in the bright position (the 45° position) between crossed Nicols is coloured and considering the interpretation of this fact. Let us notice first that, if monochromatic light of wavelength λ is used, then the wavelength in the wall will be less than this; suppose it is λ_γ for light vibrating parallel to the m.e.p. and λ_α perpendicular to this ($\lambda_\gamma < \lambda_\alpha$). Then, if the wall thickness is d, the number of wavelengths in the wall thickness will be $\frac{d}{\lambda_\gamma}$ and $\frac{d}{\lambda_\alpha}$ respectively. There will therefore be a difference in the number of wavelengths of $d\left(\frac{1}{\lambda_\gamma}-\frac{1}{\lambda_\alpha}\right)$ and it is for this reason that the wall is bright. Multiplying this by λ gives the difference in the two paths in the same units as for d.

$$p=d\left(\frac{\lambda}{\lambda_\gamma}-\frac{\lambda}{\lambda_\alpha}\right)$$

or, remembering the definition of refractive index (p. 51)

$$p=(n_\gamma-n_\alpha)d.$$

This is called the *path difference*. If the path difference is zero, then the substance is isotropic (like glass) and the object is dark in all positions. As the path difference increases, the wall becomes bright in the 45° position; but when the path difference is equal to one wavelength then the vibrations leaving the wall are in step again, just as they were on entering; they therefore reform the same vibration *PP* as entered the wall and this is extinguished by the analyser. The object is then dark at all azimuths just as if the wall were isotropic, and the same thing is obviously equally true if the path difference is 2λ, 3λ or any whole number of wavelengths.

If now white light is substituted for the monochromatic light, then in general the object will appear coloured. For assume, for instance, that the path difference is equal to the wavelength of green light; then green light, and green light only, will be completely missing from the light transmitted by the analyser and this light will then be white light

minus green, *i.e.* red. For very small path difference, small in comparison to the wavelength of light, almost equal proportions of all radiations will be lost and the object will appear grey. As the path difference increases it will appear first white, then yellow and so forth, up to red. All these colours are said to be *first order colours*. The point at which the path difference is one wavelength for green light arbitrarily marks the end of this first order. Similarly, the subsequent point at which the path difference is two wavelengths for green marks the end of the second order and so on. With increase in path difference, therefore, a steadily changing colour is observed in an object between crossed Nicols, in a regular series called Newton's Series. The colours, together with the corresponding path differences, are given in Table II, and an attempt has been made to reproduce the colours themselves for the first order in Plate III, Fig. 1.

TABLE II

The first two orders of Newton's Colour Scale (modified from Quincke)

Path difference (mμ) ($\lambda = 589$ mμ)	*Order*	*Colour between crossed Nicols*	*Colour between parallel Nicols*
0		Black	Bright white
40		Iron-grey	White
97		Lavender-grey	Yellowish-white
158		Greyish-white	Brownish-white
218		Clearer grey	Brownish-yellow
234		Greenish-white	Brown
259		Almost pure white	Light red
275		Pale straw-yellow	Dark reddish-brown
306	I	Light yellow	Indigo
332		Bright yellow	Blue
430		Brownish-yellow	Greyish-blue
505		Reddish-orange	Bluish-green
536		Red	Pale green
551		Deep red	Yellowish-green
565		Purple	Lighter green
575		Violet	Greenish-yellow
589		Indigo	Golden-yellow
664		Sky blue	Orange
728		Greenish-blue	Brownish-orange
747		Green	Light carmine
826		Lighter green	Purplish-red
843		Yellowish-green	Violet-purple
866	II	Greenish-yellow	Violet
910		Pure yellow	Indigo
948		Orange	Dark blue
998		Bright orange-red	Greenish-blue
1101		Dark violet-red	Green
1128		Light bluish-violet	Yellowish-green
1151		Indigo	Impure yellow

The use of this colour scale now offers a method of determining the extinction positions much more accurately than can be done otherwise, and, what is more important, of distinguishing between the major and the minor refractive indices. For reasons which will become obvious, the method is to interpose somewhere in the optical path of the microscope, usually between the analyser and the objective, a slice of a crystal, commonly selenite, whose path difference is such as to show a red colour of the first order (Red 1). This is placed at 45° to the direction of vibration of the polarizer (Fig. 30(*a*)) with the m.e.p. ($n_{\gamma p}$), say, lying from the upper right down to the lower left, and the field now appears red. The cell wall may now be placed on the stage and rotated. When the m.e.p.s of the wall and plate are parallel, then the path difference of (plate plus wall) is greater than that of the plate alone and the colour of the combination is higher in Newton's series than is that of the plate alone. The effect is that the wall appears blue or green, depending on its path difference, against a red background (Fig. 30(*b*)). If the two are at right angles to each other (Fig. 30(*c*)) then the colour is equally low in Newton's Series and the wall appears orange or yellow against the red background. When, however, one extinction position of the wall is parallel to *PP*, then the wall has no effect on the light and appears therefore the same colour as the field. Inspection of Table II and Plate III, Fig. 1 will show that the change of colour with path difference is particularly rapid in the neighbourhood of Red 1 and that is why this plate is used. The method of determining the m.e.p. should now be obvious. The stage is turned until the wall is the same colour as the field, and the angular position of the stage is noted. This is repeated several times and the average position obtained.* Now the stage is turned slightly clockwise. If the colour of the wall changes to blue or green (*i.e.* ascends Newton's Series, or shows an *addition colour*) then the extinction position which is parallel to *PP* is the m.e.p.: if the colour descends the series (shows a *subtraction colour*) then the position is the minor extinction position. Assuming the wall to be in the former position, then the analyser can be thrown out of the system and the stage turned until the edge of the wall is parallel to the cross wire in the eyepiece which in turn is parallel to *PP*. The angle through which the stage must be rotated then gives the angle between the m.e.p. and the cell length.

A beautiful and instructive example of the colour effects associated with structure is provided by starch grains, and a colour plate of starch

* In practice the stage is then turned through 90° and the other m.e.p. obtained. The resulting figure should be 90° removed from the first. This procedure ensures correct colour matching.

grains from potato is given in Plate III, Fig. 2. The grains are all coloured, and the variation in colour is at first sight bewildering. Careful observation of the details readily gives, however, some useful information as to their structure. Suppose attention is first confined to the smaller spherical grains. Then each grain shows a red maltese

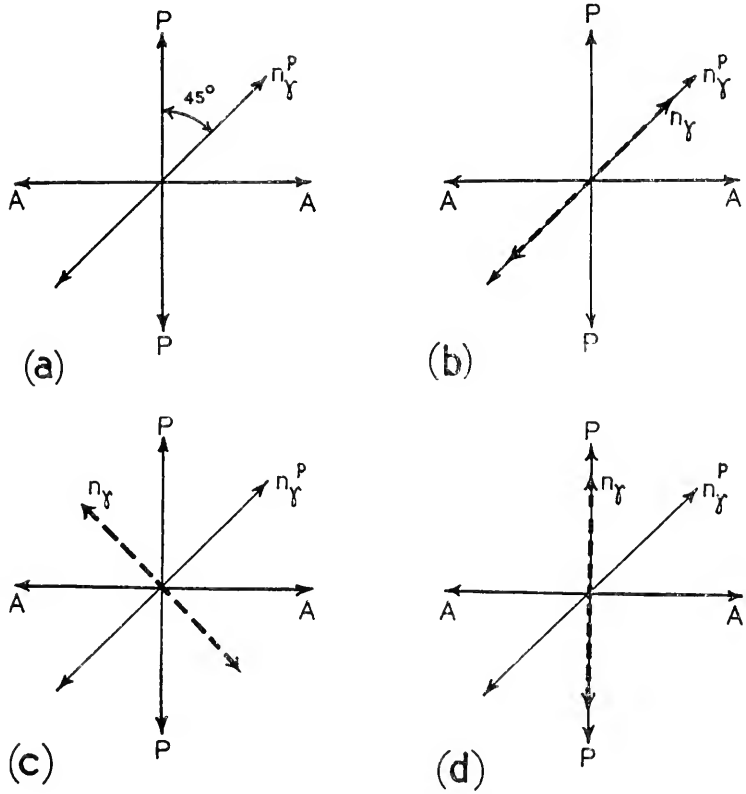

FIG. 30. The use of a colour plate in determinations of the m.e.p.

(*a*) The colour plate (Red I) is inserted between crossed Nicols in the 45° position. The field is red.

(*b*) The wall is superposed on the plate and its n_γ direction is parallel to that of the plate, when the slow vibration in the plate remains the slow vibration in the wall. Hence the path difference of (wall plus plate) is greater than that of the plate alone. The path differences are therefore additive, and an addition colour, blue or green, is shown.

(*c*) If the wall is now turned through 90° the slow vibration in the plate becomes the fast vibration in the wall, and therefore overtakes the other vibration slightly. The total path difference is therefore less than that of the plate alone, and the colour is a subtraction colour (orange or yellow).

(*d*) In an intermediate position where the n_γ (or n_α) position is parallel to *PP*, the wall has no effect on the light and the wall is the same colour as the field.

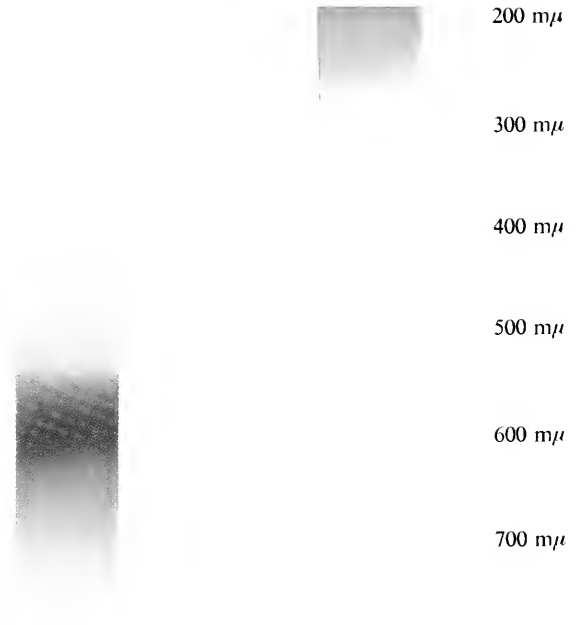

FIG. 1. The first order in Newton's colour series as shown by a quartz wedge (*a*) between crossed Nicols; (*b*) between parallel Nicols.

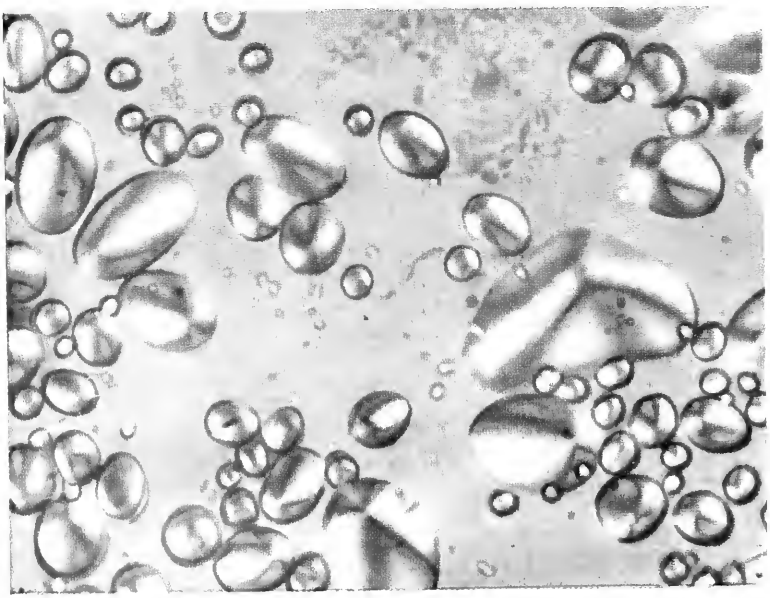

FIG. 2. The appearance of starch grains between crossed Nicols over a plate, Red I. For explanation, see text, p. 64.

[*Between pp.* 64/65

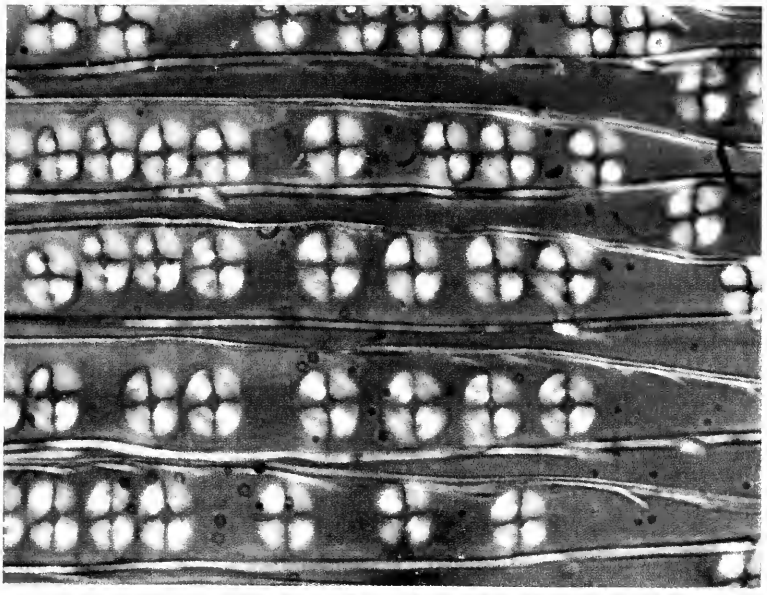

FIG. 3. The appearance of bordered pits in conifer tracheids under conditions as in Fig. 2. For explanation, see text, p. 64.

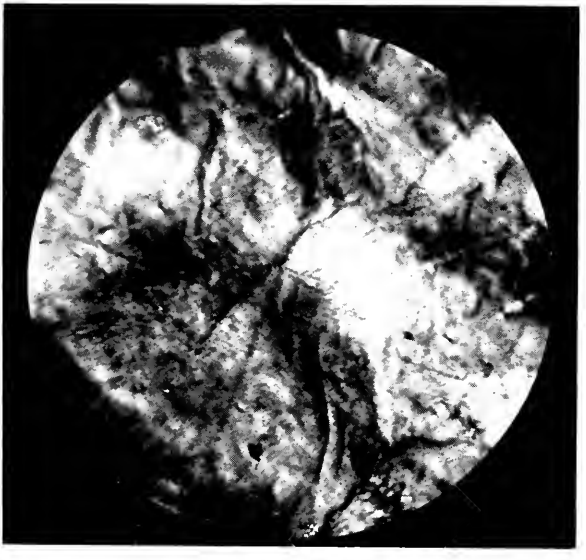

FIG. 4. A piece of Valonia cell wall under the polarizing microscope between crossed Nicols (plane of vibration of polarizer parallel to short edge of page). Note the variations in brightness over the field of view; this implies a variation in m.e.p. See text p. 95.

cross, whose arms are parallel to the directions of vibration in the polarizing prisms. The upper right and lower left sectors are coloured green, *i.e.* show an *addition* colour, while the upper left and lower right are in *subtraction* colour. Along the arms of the cross, therefore, the m.e.p. of the starch crystallites must lie either parallel or perpendicular to the arms of the cross, *i.e.* radially or tangentially in the grain. Since the upper right-hand sector is green, and the grain must be symmetrical, it follows that the m.e.p. must lie radially. Here, then, all the colour effects shown by a cell wall on rotation are manifested without rotation. It should be clear that if the grain is rotated, the cross and the sectors remain stationary. Interpretation of the larger grains is now easy. The same phenomena are shown, but they are slightly distorted on account of the eccentricity of the grain: in every case the m.e.p. of the crystallites (and therefore presumably the long molecular chains of amylose) lie radially to the lamellae. Plate III, Fig. 3 shows a somewhat similar type of structure in bordered pits of conifer tracheids. Comparison with Plate III, Fig. 2 will make it clear, however, that here the particles (this time of cellulose) lie *tangentially* to the edge of the border.

With the particular wall object examined so far, it would be found that the m.e.p. is exactly parallel to the edge of the wall. This is quite a general rule for walls seen edgeways. Since the m.e.p. represents the projection of the cellulose chain direction in the plane of the section, it follows that the chains always lie flat in the surface of the wall. This is presumably a consequence of their deposition at the surface of the cytoplasm.

Returning to Fig. 28, suppose now attention is paid to the area A, where the top wall of the cell has been removed so that a single wall is observed in face view (this can be done quite readily, see p. 116). Then it will generally be found in elongated cells that the m.e.p. is tilted to the cell length along, say, *LM*. The angle θ can then be determined. A moment's thought will add the further information that, considering the cell as a whole, the m.e.p. lies along a spiral defined by the angle θ. This must be true since:

(*a*) However the cell is cut, the m.e.p. is always tilted in the same direction or, what is actually observed, if a whole population of cells are cut in this way, then all the m.e.p.s are usually tilted in the same direction.

(*b*) If the double wall at *D* be examined, then it will be found that its m.e.p. can be determined only approximately, but it lies very nearly parallel (or perpendicular depending on whether θ is less or greater than 45°) to the length of the cell. It will be clear from Fig. 31 that this means

that the m.e.p. of the upper wall is tilted at the angle θ to cell length but in the opposite direction.

In a population of cells, therefore, a series of θs can be observed for individual cells and the average calculated. With care, each value of θ is reproducible to within $\pm 0{\cdot}5°$, and with practice something like 30–50 determinations can be made per hour. The results are recorded, as usual in statistical work, as range, average and standard error.

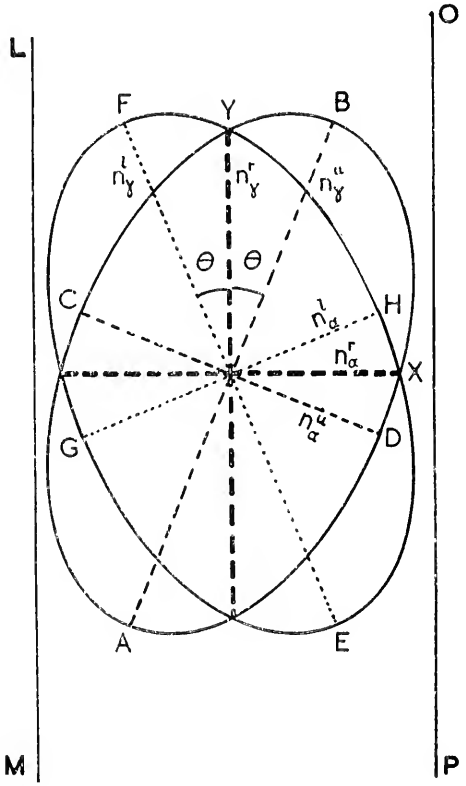

FIG. 31. *LM* and *OP* are the edges of a cell seen in face view so that the observer is looking down through two equally thick walls. *ABCD* represents the trace on the wall surface of the index ellipsoid of the upper wall, and *EFGH* that of the lower wall, the m.e.p.s being assumed to lie at the same angle to the cell length. Reasons of symmetry show that under these conditions the effective extinction positions lie along the thick broken lines. In point of fact, two superposed plates of this kind cannot be treated exactly as a single uniaxial plate; the extinction positions do not show true extinction, but represent rather positions of minimum intensity. Wherever, therefore, the "extinction" positions of a double wall lie parallel and perpendicular to cell length then the m.e.p.s of the individual walls must make the same angle to the length of the cell but on opposite sides. The organization of the m.e.p. is then spiral. A special case occurs when the angle is zero.

The significance of path difference. Birefringence

Thus far, optical methods have been shown to yield information as regards the orientation of refractive indices in a wall. It should be noted that the m.e.p. gives the cellulose chain orientation only if the wall is homogeneous in chain direction along the direction of propagation of the light, so that interpretation of the m.e.p. has to be made with care. Examples of the pitfalls awaiting the unwary will appear later in abundance. Optical methods allow us to do far more than this, however. It has been seen that if the cellulose micelles are in fact all strictly parallel to each other in a wall, then n_γ is high and n_α is low, so that $n_\gamma - n_\alpha$ is high. If the micelles are displaced from this parallel position, or if foreign bodies are introduced (even air) which are less strongly anisotropic or even isotropic, or if the amount of non-crystalline cellulose is increased, then $n_\gamma - n_\alpha$ decreases. The value of $n_\gamma - n_\alpha$ therefore gives us some information as to the precise condition of the cellulose complex. Further, reference back to Fig. 27 will show that if n_γ and n_α for the cellulose are known, and n_γ' is measured for any section, then the angle of tilt of the section to the cellulose chain direction can be calculated or, conversely if n_γ' and θ can both be measured, then n_γ can be calculated. Determination of n_γ', the major refractive index in a section, is therefore often very useful and it should be pointed out here that, since n_α is relatively constant the measurement of the difference $n_\gamma' - n_\alpha$ is equally useful. This value, which is clearly a measure of the degree of crystallinity of a wall, is known as the *birefringence* or the *double refraction* and can be determined rather easily without determining the separate refractive indices individually. This is often fortunate since, with cellulose, it may take several hours to determine one set of refractive indices whereas the double refraction of one wall can be determined in a few minutes.

It will already be clear from Table II that the path difference can be determined approximately by noting the colour of a wall when in the 45° position between crossed Nicols or, better by noting the addition and subtraction colours when rotated over a Red I plate. Neither method is capable of any great accuracy, even if only in view of the difficulty of matching colours in a microscope against printed colours, as presented in Plate III, Fig. 1, or against descriptions given in Table II. Some much more exact method is needed. In principle the method used is simple. The object to be investigated is placed in the 45° position to the vibration directions of the crossed Nicols as described above. A device called a *compensator* is then inserted in the microscope a variety of which is available though space will not allow anything but a bare

mention of them. Further details can be obtained from any of the standard text-books (*e.g.* Ambronn and Frey, Hartshorne and Stuart, Johannsen(12)), but the method can be illustrated by considering a quartz wedge. Since the birefringence of quartz is known, then the path difference at any point in a wedge made of quartz can be calculated. If the wedge is inserted in the microscope, again at 45° to the vibration directions but with the m.e.p. at right angles to that of the object under investigation, then by sliding the wedge into the microscope a point can be reached at which the path difference of the object equals the path difference of that part of the wedge lying over it. The object plus the overlying wedge has then zero path difference and is black. The point is noted therefore at which the object appears black and a previous calibration gives the path difference. With care, the object can then be turned on its side so that its thickness, d, can be measured and the birefringence, $n_\gamma - n_\alpha$ can be calculated.

Normally, however, methods based on this principle are too insensitive to be used with cell walls, since the path differences to be measured fall rather low in the first order. For cell walls a much more delicate method is necessary and fortunately this is available in a compensator devised by de Sénarmont and called after him. The method has been described by Ambronn and Frey(12) but as far as the writer is aware the theory has not been given in any formal way. This will be presented elsewhere* and at the moment attention can only be given to the method itself. In point of fact the compensator measures not the path difference but what is called the *phase difference*. This can best be understood by reference back to Fig. 13 (p. 36). If two vibrations differ in path length by λ then one has completed one whole vibration in advance of the other. Consider for a moment the vibration at any one point M (Fig. 32). The point M is vibrating along MM' with simple harmonic motion and this motion can be derived by describing a circle with MM' as diameter. Suppose a point P is then chosen on this circle and the perpendicular from it to the line MM', Pm, be constructed. Then if P is allowed to move round the circle with constant velocity the point m moves along MM' in the manner required. If the point moves from M to M' and back again,

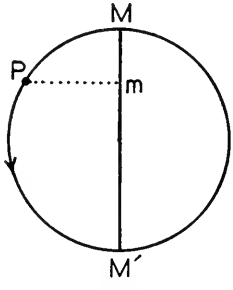

FIG. 32. For explanation, see text.

* In *Problems and Methods in Plant Biophysics* to be published by Elsevier, Amsterdam.

this corresponds to one complete vibration, *i.e.* to one path length of λ. During the same time the point P makes one complete revolution, *i.e.* passes through 2π radians. Hence,

a path difference of λ=a phase difference of 2π.

Therefore,

a path difference of $(n_\gamma - n_\alpha)d$=a phase difference of $(n_\gamma - n_\alpha)d \cdot 2\pi/\lambda = \phi$.

This is what is measured by the de Sénarmont compensator. In use, the object is placed at the 45° position as usual, and illuminated by sodium light. A plate of mica, cut so that its path difference is exactly one quarter of a wavelength is then inserted in the light path between the object and the analyser, with its m.e.p. parallel to the direction of vibration of the polarizer. Now if the analyser is turned it is found that the brightness of the object first diminishes, passes through a minimum (the intensity of the field and therefore approximately zero) and increases again. The angle through which the analyser has to be turned to make the intensity a minimum is numerically equal to one-half of the phase difference required. It should be noted that since therefore a rotation of 180° is equivalent to a path difference of one wavelength, the method is a very sensitive one. Once the phase difference is known, the birefringence can readily be calculated from the above equation.

Dichroism

Before leaving optical methods of structure determination, mention must be made of a phenomenon associated with polarization in some crystals since, although cellulose itself does not show this phenomenon, it can be dyed with stains which do, and this can give useful information. So far, attention has been confined to anisotropic bodies in which the absorption of light in the body is the same for both directions of vibration. Some crystals, however, show a greater absorption for some, or for almost all, wavelengths in visible radiation for light vibrating parallel to one or more of the axes of the index ellipsoid. Such crystals are said to be *pleochroic*; here we shall deal with the simplest case in which the absorption is greater in only one direction, a phenomenon called, therefore, *dichroism*. Some of the stains used customarily to stain cellulose show this phenomenon, notably iodine and congo red. Here we shall confine our attention to iodine. If a cellulose wall is purified, *i.e.* the incrusting substances such as lignin removed, and is stained in iodine and 70% sulphuric acid, the cellulose can be seen to turn blue both in bulk to the naked eye and in single cells under the microscope. Suppose a wall such as *BC*, Fig. 28, is stained and examined

under the microscope over a polarizing prism, the analyser being removed. The wall is blue only in certain azimuths. When the wall is arranged to lie parallel to the direction of vibration of the light then it is colourless; when at right angles to this it is blue. This again, therefore, gives a ready method for the determination of chain direction. In general, for light vibrating in any direction in the plane including the major axis of the index ellipsoid, Fig. 27(*a*), the wall is colourless. More than this, however, for if the dichroism is not blue-colourless but blue-yellow then this means that the cellulose preparation is impure; and if it is dark blue-light blue then this means that the cellulose micelles are considerably displaced from their parallel position, *i.e.* they possess a considerable degree of *angular dispersion.* Measurement of the intensity of the light transmitted along the two directions can be used to give a semi-quantitative measure of this dispersion(26). It should, however, always be remembered that iodine staining here involves a swelling of the wall in concentrated sulphuric acid. This itself will tend to disturb the arrangement of the micelles. Similarly in some dyes which show polarized fluorescence the angular dispersion can be estimated(27).

This dichroism resulting from iodine staining can be traced to the similar dichroism of iodine crystals. It seems that the staining involves the growth, in the "intermicellar spaces", of iodine crystals of submicroscopic dimensions which lie parallel to the micellar direction.

CHAPTER V

The Structural Features of Cellulose and the Spatial Relationships of the Incrusting Substances

THE X-RAY diagram of cellulose indicates, as we have seen, that this substance is built up of long molecular chains of anhydroglucose residues at least 600 Å. long which, over some part of their length, are arranged regularly in a crystalline lattice. It now becomes necessary first to see how far this picture is in harmony with the behaviour and physical characteristics of cellulose, and this chapter will therefore be devoted to those properties of cellulose which are common to all celluloses wherever they occur.

Perhaps attention may first be called to the ready understanding of the insolubility of cellulose, built up though it is of soluble glucose units, and its lack of a melting point. Here an analogy can be drawn with the homologous series of the paraffins. Methane is a gas which condenses to a liquid only at a very low temperature. Ethane, the next in the series, is also gaseous but can be liquefied at a much higher temperature. Passing through propane and butane up to octane we pass through gases with higher and higher liquefying temperatures to a substance which is liquid at room temperature. Higher still in the series, the substances become solid in the familiar paraffin wax. At first sight this is rather remarkable since each member of the series differs from the last only by the addition of another $—CH_2$ group. A moment's thought, however, will make the matter clear. A liquid evaporates in so far as the kinetic energy of the molecules due to their heat movement is higher than the energy binding one molecule to its neighbours. The energy which has to be put into a molecule to cause the separation from its neighbours, and therefore the boiling point of the liquid, depends therefore on the bond energy between the molecules. Now in methane any two molecules are held together only by single secondary valences. The energy concerned is of very small magnitude and a very little heat motion suffices to keep the molecules apart. As the series is ascended, however, since the molecule is increasing in length by the addition of further $—CH_2$, each of which will exercise a secondary valence attraction on neighbouring molecules, the bond energy increases until with octane,

where the valence forces are some eight times greater than with methane, the substance is liquid at room temperature. By an extension of this argument it is easy to see why higher members of the series finally become solid—the molecules are firmly bound together by the co-operation of many small forces. Similarly with cellulose. The anhydroglucose units in the chain are held together by C—O—C links whose binding energy (*i.e.* the energy required to separate the molecules) is of the order of 70 kilocals. per gm. mol. Laterally, however, the chains are held together by hydrogen bonds between neighbouring —OH groups, whose energy is in the neighbourhood of only 5 kilocals. per gm. mol. Correspondingly, single molecules in a sugar crystal can readily be separated from each other, *i.e.* the crystals are readily soluble (due to the mutual attraction of the hydroxyls in glucose and in water) and are fusible. In cellulose, however, where very many hydrogen bonds may be expected to cooperate in holding two chains together, and where many of the hydroxyls "satisfy" each other within the crystalline lattice, the substance is swellable but not soluble; and, since the energy required to separate many hydrogen bonds uniting two chains is greater than the energy of a single C—O—C link, the substance burns or chars (involving a breakdown of C—O—C) before it melts. In these and in other physical properties, cellulose thus behaves as it does because it consists of long molecular chains. Let us review very briefly the various other lines of evidence which indicate long chain length, and the various attempts which have been made to estimate chain length.

The molecular weight of cellulose

With polymeric substances, among which cellulose must be classed, it is not always easy to say precisely what is meant by the molecule, and therefore the allocation of a molecular weight is correspondingly somewhat arbitrary. With two-dimensional lattices such as occur, for instance, in graphite, the "molecule" should properly refer to the whole sheet of atoms in a plane; and with three-dimensional lattices such as diamond or silica the "molecule" is equally clearly the whole of the atoms within any one region of perfect crystallinity, *i.e.* the crystallite. In neither of such cases is it easy, or often even possible, to define the molecule. With linear polymers, on the other hand, the problem is much easier and with cellulose the molecule can readily be defined as one single chain of anhydroglucose units, however difficult the determination of the corresponding molecular weight may prove to be. That is not to say, of course, that the chains of cellulose are likely to be of the same length; on the contrary it is highly probable, on *a priori*

reasoning, that the chains will differ, perhaps widely, in length. Whereas, therefore, in simple inorganic molecules we are accustomed to think in terms of a molecular weight which can be determined with a considerable degree of accuracy, and which can be considered to represent the weight, relative to hydrogen, of each and every molecule, in cellulose we must be prepared to think in terms of an average molecular weight. This carries with it two implications. If the molecular chains of cellulose do vary considerably in length (as in fact they do) then firstly, different methods of determining molecular weights will give different results and, secondly, the physical behaviour of two celluloses with the same "molecular weight" may be different if the range in molecular weights is different. Before discussing these points it will be as well briefly to look into the methods used for molecular weight determination in cellulose.

The determination of molecular weights

It should be noted at the outset that molecular weight determinations can be made only in solution, and in substances like cellulose this imposes strict limits on the accuracy with which the molecular weight of untreated cellulose can be estimated. This is true since there is no solvent known for cellulose which does not cause some degenerative breakdown (*i.e.* decrease in chain length) during solution. Cellulose is dissolved usually in cuprammonium; or it may be nitrated and the nitrocellulose dissolved in acetone, etc., and clearly the resulting chain length will depend on the treatment and the care with which the various operations involved are carried out.

The actual methods used can be classed as physical and chemical (or analytical) and these will be discussed in turn. Some results of the various methods are collected in Table III, where the striking variations in the estimated value of the molecular weight bears testimony to the difficulties involved. The results in Table III are given in terms of the "degree of polymerization" *i.e.* the number of glucose residues united in one chain, since this gives a clearer mental picture of the condition arising in cellulose than would a statement of the molecular weight itself. The actual molecular weight can be derived by multiplying the figure given here by 162.

(*a*) *Osmotic pressure determinations.*—It should be clear that since osmotic pressure depends only on the number of particles of a solute in a solution and not on their size, then measurement of the osmotic pressure of a solution of known concentration (gm. per litre) will enable the molecular weight to be determined. The method is of general

TABLE III

Degree of polymerization (number of glucose units per chain) in cellulose from various sources

Source	*Method*	*Degree of Polymerization*	*Authority*
Ramie fibres	End group (aldehyde)	175	Bergmann and Machemer (1930)
Ramie fibres	X-rays	>200	Hengstenberg and Mark (1929)
Wood pulp	End group	115	Bergmann and Machemer (1930)
Wood fibres	X-rays	>120	Hess, Trogus, Akim and Sakurada (1931)
Cotton	End group (Tetramethylglucose)	100–200	Haworth and Machemer (1932) Haworth and Hirst (1933)
Cellulose	Ultracentrifuge	1800	Kramer and Lansing (1933)
Raw cotton	Viscosity	2700	Staudinger and Mohr (1937)
Cotton	End group (Tetramethylglucose in nitrogen)	695	Haworth, Montanna and Peat (1939)
Cotton *Nettle fibre* *Ramie fibre* *Wood pulp*	Ultracentrifuge Ultracentrifuge Ultracentrifuge Ultracentrifuge	9200 10800 11300 2900	Gralen and Svedberg (1943)
Raw cotton	Viscosity in nitrogen	10000– 15000	Galova and Ivanov (1945, 1946)

importance in the field of high polymers particularly since it possesses a sound thermodynamic basis. With substances of low molecular weight it is often preferable, if only on account of the simplicity of the experimental technique, to determine, not osmotic pressure itself, but the other, related, colligative properties such as the depression of the freezing point or the elevation of the boiling point. These cannot be used here, however, for a very simple reason (29(*c*)). Suppose we have a substance of molecular weight, say 68,000 (which is much lower than that of most normal celluloses) and prepare a solution of 10% concentration. Assuming that the depression of the freezing point for water containing 1 gm. mol. of solute in 1000 gm. of water is 1·86° C., then the depression for this solution would be (10/68,000)1·86=0·00027° C. Not only is this too small to be measured with any accuracy, but a small contamination with a low molecular weight substance would cause a depression of a similar magnitude and therefore vitiate any result obtained. On the other hand, again assuming ideal behaviour, the osmotic pressure itself would be about 34 mm. of water. Not only can this be measured accurately but the effect of low molecular weight contaminants can be eliminated.

Ideal osmotic behaviour can be expressed by the van't Hoff equation

$$P=(c/M)RT,$$

where P is the osmotic pressure, c the concentration in gm. per litre, M the molecular weight, T the absolute temperature and R the gas constant. Obviously if this equation is valid, a plot of P against c should be a straight line passing through the origin. With polymers this is generally not so; and it must be remembered that the van't Hoff equation is in fact valid only for low concentrations, *i.e.* in solutions such that there is no interaction between the dissolved particles themselves. With substances of high molecular weight, *i.e.* with bulky molecules, the concentration required to fulfil these conditions is likely to be very small indeed, much smaller than that necessary for the more normal low molecular weight substances. Correspondingly, it is found that at low concentration a plot of P against c becomes approximately linear. At higher concentrations the relation is of the form

$$P=(c/M)RT+kc^2,$$

k being a constant, or

$$P/c=RT/M+kc.$$

A plot of P/c against c is therefore approximately a straight line, and the intercept on the ordinate (P/c axis) at the point $c=0$ is equal to

RT/M, from which M can be calculated. Pressure measurements are therefore made over a range of low concentrations and the curve extrapolated to zero concentration so that

$$\underset{c\to 0}{\mathrm{Lt}}\ \frac{P}{c}=\frac{RT}{M}.$$

It is particularly to be noted here that osmotic pressure determinations, depending as they do on the *number* of particles in solution, give a *number average* molecular weight, i.e.

$$M_n=\frac{\Sigma n_i M_i}{\Sigma n_i}.$$

where n_i is the number of gm. moles of molecular weight M_i and the summation is taken over all values of i.

(*b*) A second method for the determination of high molecular weights and one which was, in fact, devised precisely for that purpose, involves the ultracentrifuge of Svedberg. Actually there are two distinct methods, the method of *sedimentation equilibrium* which again has a sound thermodynamic basis, and that of *sedimentation velocity*. This latter, in view of the fact that it does not involve equilibria, has naturally no thermodynamic basis but the theory underlying the method is now unquestioned. These may be considered briefly in turn.

(1) *Sedimentation equilibrium*

(*b*) The principle of this method can perhaps best be grasped by considering the equilibrium attained in a column of air under the earth's gravitational field. The molecules of gas will tend to separate out and are prevented from doing so only by the thermal agitation of the molecules. This leads to a variation in the properties of the atmosphere with which everyone is familiar. Thus, considering unit cross-section, the decrease in pressure dp, for a small increase in height dh, is given by

$$-dp=g\varrho dh,$$

where ϱ is the mean density of the gases at height h. Assuming the gases to be perfect, then

$$\varrho=Mp/RT,$$

whence

$$-dp/p=(M/RT)g\,.\,dh,$$

which integrates to

$$\ln\,(p_1/p_2)=(Mg/RT)(h_2-h_1), \qquad ..(1)$$

where p_1 is the pressure at height h_1, and p_2 that at height h_2.

Equilibrium in the ultracentrifuge is precisely analogous to this. The centrifugal field takes the place of gravity and is very high (about 10,000g) in order to bring measurable effects within small compass. At equilibrium, in which a balance is obtained between sedimentation and diffusion, there is a particular distribution of concentration which can be treated in a way quite analogous to the above treatment of gaseous pressure. Experimentally, the solution is held in a cell with transparent windows and is spun at speeds up to about 15,000 r.p.m. The contents of the cell are examined optically by methods described elsewhere (28), and the spinning continued until the distribution of concentration remains constant. Then, corresponding to equation (1) above,

$$dc/c = M(1-v\varrho)\omega^2 x \,.\, dx/RT, \qquad ..(2)$$

where x is the distance of the point of observation from the centre of rotation ($\equiv h$ in equation (1)), $\omega^2 x$ is the angual acceleration ($\equiv g$) and $M(1-v\varrho)$, where ϱ is the density of the solvent and v is the partial volume of the solute (or $M(\varrho_{\text{solute}}-\varrho_{\text{solvent}})$), replaces M. On integration

$$M = 2RT \ln (c_2/c_1)/(1-v\varrho)\omega^2(x_2^2-x_1^2).$$

This equation again, as in the osmotic case, applies only to low concentration.

(2) *Sedimentation velocity*

(*b*) In this method, the centrifugal field is made so large that opposing diffusion is negligibly slow and no equilibrium is reached. Normally fields of 100,000 to 300,000g suffice, though Svedberg has used fields up to 500,000g. In principle, the theory is very simple. If a molecule of weight M is moving with velocity dx/dt under a centrifugal acceleration $\omega^2 x$, then equating the forces causing and opposing motion, we have

$$M(1-v\varrho)\omega^2 x = F \,.\, dx/dt,$$

where F is the frictional constant per mole, or, defining a *sedimentation constant*, s

$$s = (dx/dt)/\omega^2 x,$$
$$M = Fs/(1-v\varrho). \qquad ..(3)$$

For spherical particles, the frictional constant was given by Stokes as

$$F_0 = 6\pi N\eta r,$$

N being Avogadro's number, η the viscosity of the solvent (see below) and r the radius of the particles. This cannot, however, normally be

assumed, and is certainly not applicable to the linear molecules of cellulose. In these cases the frictional constant may be identified with that occurring in the equation

$$D=RT/F,$$

where D is the diffusion coefficient of the same solute. Substituting this value in (3),

$$M=RTs/D(1-v\varrho). \qquad ..(4)$$

Experimentally the method involves the observation of the movement of the boundary between solution and pure solvent as this moves down the tube. This is done by optical methods into which space will not allow us to go; they can be obtained from any of the standard works on sedimentation. If the solution contains two types of sedimenting particles, then these will move down the tube at different rates, giving two boundaries which will move apart. There are naturally limits to the number of components which are resolvable, but nevertheless this method does yield valuable data on the composition of the more important fractions in a colloidal solution.

(*c*) *Viscosity determination.*—During the past few years a great deal of attention has been paid to the possibility of determining molecular weights from the viscosity of solutions, particularly in view of the ease with which viscosity determinations can be made. As with the other two methods described, this has involved the development of theoretical approaches into which we cannot go here. Again these can be obtained from the relevant literature. Briefly the viscosity of a liquid is that property which is responsible for the internal resistance offered to the relative motion of different parts of the liquid. In a solution, this resistance will clearly be affected by the features of the solute, including the size of the dissolved particle. In order to define the *coefficient of viscosity* it should be recalled that for pure liquids Newton assumed the shearing force, τ, between two parallel planes of liquid in relative motion to be proportional to the area of contact and to the velocity gradient dv/dx between them, giving his well-known relation

$$\tau=\eta dv/dx \,.\, A. \qquad ..(5)$$

The factor η is then a constant called the coefficient of viscosity, a property of the liquid and independent of the conditions used for its determination. The determination may be made by any one of a number of methods.

If, for instance, the liquid is caused to flow along a capillary tube l cm. long and a cm. diameter under a pressure difference of P between

the two ends of the tube, then the volume of liquid passing down the tube per second is related to the viscosity by the well-known Poiseuille's equation,

$$V=\pi Pa^4/8\eta l, \qquad ..(6)$$

so that η can readily be determined. For most pure liquids and for many solutions, Newton's Law (eqn. 5) has been completely confirmed. For most colloidal solutions, however, the proportionality between shearing force and velocity gradient is absent at low velocity gradients, the "apparent" viscosity decreasing as the velocity gradient is increased. This is true for solutions of cellulose. The anomalous behaviour tends to disappear for solutions of low concentration, since it is due in large part to an interaction between the dissolved particles of a type not accounted for in the simple Newtonian Law. Thus again the practice is to make determinations over a range of small concentrations and to extrapolate to zero.

As regards the connection between molecular weight and this limiting viscosity, mathematical relationships for spherical particles and for ellipsoidal particles have been worked out, but as yet there is no sound theoretical basis for treatment of linear polymers such as cellulose. For the moment, therefore, recourse has to be made to empirical relationships. The one most frequently used was proposed many years ago by Staudinger who based his considerations on the viscosity of medium and low molecular weight polymers, whose molecular weights were known from osmotic and other determinations. He proposed a relation of the type

$$\eta_{sp.}/c=K_m \,.\, M,$$

where $\eta_{sp.}$ is defined as (η/η_0-1), η being the viscosity of the solution and η_0 that of the pure solvent, and is called the *specific viscosity*. Later, in view of the variation of $\eta_{sp.}$ with c (the concentration), this was modified to

$$\operatorname*{Lt}_{c\to 0} \eta_{sp.}/c=K_m \,.\, M.$$

For these lower molecular weight polymers, he showed that K_m can be regarded as a constant for any one polymer-solvent system. Thus, for cellulose, a constant K_m can be derived by noting the viscosity for glucose, cellobiose, cellotretrahose, cellohexose, etc., when, on the assumption that K_m retains its value constant however many glucose units are bound into a chain, the molecular weight of cellulose can be determined by a measure of its viscosity in solution in cuprammonium. Criticism was soon levelled at this method, largely in terms of the validity

of the extrapolation to high molecular weights, and undoubtedly such criticisms were, in the main, valid. The quantitative application of the Staudinger Law is therefore open to doubt, though it certainly provides a ready method of estimating molecular weights roughly, and is used for that purpose. More recently Flory and Alfrey, Bartovics and Mark have used a different equation for higher molecular weights ($>10{,}000$) (see 30). This takes the form,

$$\operatorname*{Lt}_{c\to 0} \eta_{sp.}/c = K \,.\, M^{a}.$$

Neither Staudinger's relation nor this new one can, however, be regarded as adequate to cover the whole range of molecular weights, and it seems unlikely that any simple relationship will meet all requirements.

According to Staudinger's treatment, a long chain will make a bigger contribution to the viscosity than will a short chain, and the molecular weight calculated is usually thought of as a *weight* average,

$$M_w = \frac{\Sigma w_i M_i}{\Sigma w_i},$$

where w_i is the weight fraction of component i. It is obvious that for any system which contains more than one chain length $M_n < M_w$ and the ratio M_w/M_n can be used as an indicator of the degree of polydispersity.

(*d*) *Analytical methods.*—Of these methods the most successful has been the "End Group Method" which has been applied to many linear polymers. For cellulose, it was first applied by Haworth and Machemer (31). Referring back to the structural formula for cellulose (p. 46) it will be clear that one terminal group per chain will have hydroxyls available for substitution in the 2, 3, 4 and 6 positions, whereas any glucose residues which are not terminal can be substituted only in the 2, 3 and 6 positions. The method was therefore to methylate the specimen under the mildest possible conditions, followed by hydrolysis into the substituted glucose residues and the determination of the proportion of 2, 3, 4, 6, derivatives among the more abundant 2, 3, 6 derivatives. Other methods are also available, *e.g.* the use of reducing power, but the principle is the same. It is, of course, now clear that this method gives a minimum figure since even under the mildest treatment some degradation of the cellulose chains must occur. Several years later, in fact, using an improved technique, Haworth and his collaborators obtained much higher figures which are also included in Table III. The

"End Group Method" clearly yields a number of average molecular weight as in the case of the osmotic method.

The molecular weight of cellulose and modifications of the original Micellar Hypothesis

Inspection of Table III brings out immediately the striking fact that over a period of only ten or eleven years the estimate of the molecular weight of cellulose has increased some fifty times. Historically the figure proposed by Haworth (100–200 residues in a chain) following so soon after the estimate of 600 Å. (=approximately 120 glucose residues), offered apparent confirmation of the original structure envisaged in terms of discrete micelles; and it was possibly this striking agreement which led at the time to the tacit assumption in some quarters that the X-ray determination gave a figure of 600 Å., whereas of course this was given as the *minimum possible* figure. This misconception, curiously enough, still lingers here and there in the literature.

The succeeding estimates of Staudinger, even though in error by a considerable margin, were so very much greater than either of these that it became clear that, *again assuming a micelle length of* 600 Å. the molecular chains of cellulose must partake in the construction of more than one micelle, and the "fringe micelle" hypothesis was born (Fig. 25(*a*)). Remembering, however, that the X-ray determination implies only that the chains are arranged parallel to each other in a lattice over lengths of *at least* 600 Å., then the structure proposed by Meyer (Fig. 25(*b*)) is equally in harmony with the facts; indeed, on the evidence before us now, it is only in so far as the original estimate of Haworth and Machemer suggests points of weakness spaced some 100–200 units along the chains, that the fringed micelle hypothesis is any longer tenable.*

The lateral dimensions of the micelle are in somewhat the same position. The value of 50 Å. given by X-ray methods can mean that the chains of cellulose lie parallel to each other, and spaced exactly the same distance from each other, only over regions of this size. The space in between could be filled with chains of cellulose lying in more random fashion, and even strictly parallel to each other so long as they are not arranged regularly the same distance apart. There is no evidence here, therefore, for discrete particles, and the conceptions of structure as pictured in Fig. 25 are so much more in harmony with the tensile and

* Recent observations by Svedberg and his colleagues (74) have shown that cellulose (cotton) can, however, be hydrolyzed by 2·5 *N* H_2SO_4 to give particles some 600 Å. long and 50–100 Å. wide, (*see* p. 90).

other properties of cellulose that there can be no doubt but that the more modern view is in essence correct. Thus the tensile strength of cellulose compares very favourably with that of metals (Table IV) and this could hardly be so if the substance were composed of isolated micelles.

TABLE IV

Tensile Strength of Cellulosic and other Materials

Material	*Tensile strength* (kg./mm.^{2})
Wrought iron	>20
Hardened steel wire	up to 170
Copper wire	up to 40
Aluminium	up to 10
Lead	up to 3
Silk	35
Cotton	28
Irish flax	100
Viscose rayon	25
Viscose rayon (well orientated)	80
Acetate rayon	18–20
Acetate rayon (well orientated)	up to 100

Cellulose occurring in plant cell walls can therefore be thought of rather loosely as a two-phase system. In one phase the chains are arranged in a regular crystal lattice into which water cannot penetrate, and other substances penetrate only with difficulty, and in the other the assembly of chains is non-crystalline. Into the latter, water can penetrate easily (causing swelling) and other substances rather easily depending on their molecular size, electric charge and so on. It should be obvious, therefore, that while the anisotropy of physical properties depends on the degree of alignment of the cellulose chains (and therefore on the orientation, angular dispersion and so on of the crystalline fractions) the precise value of any property in any particular direction in a piece of cellulose will depend to a large extent on the non-crystalline fraction. Thus such properties as swelling, moisture absorption, density, extensibility, rigidity and time effects on mechanical properties will depend to a large extent both on the degree of alignment in the non-crystalline fraction and on the relative amount of such non-crystalline material present. The importance of this has perhaps not been stressed in the botanical literature as it has in the technological, but it must equally be of importance.

Such a division of the cellulose matrix into two phases is, however, quite arbitrary; and it is certain that between the rigidly spaced chains within a micelle and the most randomly oriented chains which may occur in the larger spaces, there are all degrees of order. It is therefore

impossible at present to estimate the degree of randomness in the non-crystalline fraction of the wall. It is, in fact, by no means certain that any of the numerous methods at present used (19, see references in 32) to estimate the *amount* of non-crystalline cellulose give values of any precision. For one thing, it cannot be expected that two different methods will give the same answer. Thus chemical methods which involve removal of the non-crystalline fraction may well give figures which are too low (if only the more loosely arranged chains are removed) or too high (if the less random chains are also attacked, since this may also involve some of the chains on the outside of the crystallites). Physical methods will yield results which are therefore at first sight more reliable, but these again will depend on the property chosen as basis. Thus density determinations will give a figure which will be a function of the looseness of packing; refractive index determination will depend both on the looseness of packing and on the degree to which the chains outside the crystallites proper are arranged parallel; and estimates by the X-ray method used by Hermans will obviously include in the non-crystalline fraction all chains which are not regularly spaced, whether they are parallel or not.

The Intermicellar System

Nevertheless it is possible to speak roughly of micelles and intermicellar spaces provided always that the implications of these terms are clearly understood, and this is especially useful perhaps when dealing with raw botanical material. Most, if not all, of the stains used in dealing with cell walls stain the intermicellar material and not the micellar fraction. Staining methods were in fact used quite early in an attempt to estimate the relative surface area of the micelles at the time when these were thought of as discrete particles. Thus the absorption of methylene blue, impregnation with gold and silver and even the adsorption of water have all been shown to give results in general agreement with the existence in cellulose of crystallites about 50 Å. or 60 Å. in diameter (33).

In some natural celluloses, and in many celluloses under special conditions, it is in fact probable that intermicellar spaces occur in the original sense of the word, *i.e.* spaces free of cellulose. Thus in the heavily silicized haulms of grasses, Frey-Wyssling showed many years ago (34) that, if the cellulose is removed, then the remaining matrix of silica behaves optically as though it were permeated by a series of narrow channels lying parallel to each other. This picture arose in virtue of the fact that such a matrix showed the form-double refraction mentioned

on p. 56 in such a way that the matrix was optically isotropic only in a liquid whose refractive index was the same as that of silica. Here, then, must have been a clear-cut topographical separation between cellulose and silica. Similarly the same worker showed more recently that in fibres wherein silver particles are induced to grow, the silver particles range in size from very small (say 10–15 Å. wide) up to 100 Å. or more, and since these spaces are filled with silver they can hardly be considered to contain any cellulose chains at all. It would seem, however, quite certain that in this latter example the growth of the larger silver particles at any rate had involved the displacement of cellulose chains, so that the size of the particles is no guarantee that empty spaces of this size occurred in the original untreated cellulose.

The distribution of the incrusting substances

From the botanical point of view it is of interest to notice the distribution of the incrusting (*i.e.* non-cellulosic) substances in the wall in terms of this roughly two-phase conception of cellulose organization. Since the lattice energy of the crystalline fraction is so high that even water does not penetrate, then it might be expected that non-cellulosic substances in cell walls would be found outside the crystalline regions, unless they happened to be such as to form, as it were, "mixed crystals" with the cellulose. This is found, in fact, to be the case.

Just as the removal of water from native cellulose has no effect on its X-ray diagram (and therefore no effect on the crystalline lattice) so the removal of lignin from lignified material has no appreciable effect. This is illustrated in Plate II, Fig. 3, for hemp fibres. The lignin must therefore occur outside the crystalline regions (35). Pectin is in somewhat the same position; in fact this substance even occurs in, for instance, collenchyma cells in separate wall layers which are almost free from cellulose (36). This positioning of lignin in particular around the crystalline fraction calls to mind the fact that in lignified material, such as wood, it is difficult to obtain a positive (blue) reaction for cellulose, on treating with iodine and sulphuric acid, until the lignin is removed. On the hypothesis of discrete micelles with real surfaces this was readily understandable in terms of a complete covering of the micellar surface by lignin. On the more recent modification of this hypothesis, a similar interpretation is, however, still possible if we assume that the chains of cellulose responsible for the reaction occur somewhere in the transition region between the crystalline (in the X-ray sense) and the non-crystalline fractions.* This particular location would also appear to be

* *i.e.* possibly where the chains are still parallel but not spaced regularly.

required by the dichroism of fibres dyed in this way, for failing this the assumption has to be made that the lignin actually occupies the place otherwise taken by the iodine. In view of the resulting dichroism of fibres stained with iodine and with, for instance, congo red (where it has been shown by an X-ray method that the elongated crystals of dye lie parallel to the micelle direction) this leads to the suggestion that the chains in the intermicellar region are still more or less parallel to each other. The same suggestion has already been put forward for sisal fibres following interpretation of their birefringence(37).

The position of the cellulosans is somewhat more difficult to define, Some years ago Norman(38) concluded on chemical grounds that the association between cellulose and xylan was much closer than that between cellulose and lignin, and this seemed to receive full support from the later discovery that removal of xylan from high-xylan fibres apparently rendered the cellulose more crystalline(35(*a*)). Again, removal of xylan from Angiosperm wood or of mannan from Gymnosperm wood has been shown to result in a decrease in the crystallinity of wood as judged by the X-ray diagram. It could therefore reasonably be concluded, since xylan is built up from xylose in much the same way as cellulose is built up from glucose, that the chains of xylan occurred inside the cellulose micelles and caused there little lattice distortion. More recently, however, other evidence has appeared which makes this interpretation less attractive (39). Thus it has been shown by Brims that if fibres containing xylan are treated even with very dilute caustic soda to remove some of the xylan, then the cellulose in the residue has a shorter average chain length. Since it seems very unlikely that the caustic soda could penetrate the crystalline cellulose to any marked extent, it seems most likely that some, at any rate, of the xylan is associated with the non-crystalline fraction even though it does appear to be associated with the cellulose in the same molecular chains. Even more critical, however, is the statement by Hirst that carefully prepared samples of xylan are composed of molecular chains which are branched. If this is substantiated, then it would be certain that xylan does not partake in the structure of the crystalline fraction of cellulose, and the earlier evidence would need reinterpretation. The last word has not been said here. It will be seen later that the relationship between cellulose and xylan may be vital to an understanding of some cell wall phenomena.

Micelle aggregates. The electron microscope

During the past 100 years or so, repeated attempts have been made to demonstrate in cell walls the presence of particles much larger than

the vaguely delimited micelles to which reference has so far been made (40). Many of these represented little more than indulgence in mere speculation but one of them, that cellulose consists of "fibrils" (*i.e.* long slender threads) which can be made microscopically visible, has persisted since the days of Grew and seems to be based on very firm supporting evidence. A considerable number of investigators have reported, from time to time and for a wide variety of cell types, that the walls can be broken down mechanically into fine threads about $1{\cdot}4\,\mu$ thick which again in turn can be shown to consist of finer threads some $0{\cdot}1$ to $0{\cdot}4\,\mu$ thick. This can readily be demonstrated with any elongated cell particularly if it is first treated with sulphuric acid or caustic soda. It has always seemed possible, however, that the development of such fibrils provided evidence for nothing more than lines of weakness to chemical and physical attack. Bailey and Kerr (47(*a*)) showed some time ago that the interpenetrating matrices of cellulose and lignin in wood cells grade down to the very limits of microscopic visibility and they suggested that the so-called fibrils were nothing more than dissected fragments of such a network. It is impossible by any ordinary method to distinguish between these two possibilities.

With the advent of the electron microscope, however, the possibility of making a critical observation in this field became obvious. It is assumed that the electron microscope has been so popularized as to give readers sufficient acquaintance with electron microscopy to know what it is all about, and it is not proposed here to attempt any description of the instrument or to enlarge upon the underlying theory. Perhaps, however, a few words by way of introduction may be of service.

Just as the light microscope is a device whereby optical magnification may be achieved to a degree impossible to the unaided eye, so the electron microscope is designed to achieve greater useful magnification than can be attained in the light microscope. *Useful* magnification, because there is, of course, no limit to the magnification which may be made by optical means of a photograph taken under the light microscope. Above about $1000\times$, however, such magnification serves no useful purpose; for clearly, and without going into the theory of light too deeply, no optical instrument could distinguish detail smaller than the wavelength of the light used, and once detail of this order is made visible, no further magnification can bring out finer detail. More formally, the diffraction theory shows that the resolving power (*i.e.* the minimum distance whereby two small particles may be separated and still remain separately visible) is given by

$$0{\cdot}61\lambda/N.A.,$$

where *N.A.* is the numerical aperture, *i.e.* the sine of half the angle subtended by the objective in the object plane multiplied by the refractive index. When it becomes imperative to observe much finer detail, then either other methods must be used (X-ray analysis being an outstanding example) or radiation of smaller wavelength must be used (this is, of course, the basis of the use of X-rays, but then no image can be *seen* since X-rays cannot be focused by lenses). This is one of the reasons why so many laboratories throughout the world have been interested in the construction of the ultraviolet microscope, using light of wavelength of the order of 2500 instead of 4000 Å. Electron microscopes go very much further. We are now familiar with the idea that electrons, though originally formulated as material particles, also have the properties of radiation, and that the wavelength of the radiation concerned is very small indeed, of the order of 0·05 Å., dependent on the energy of the electron. The relation between wavelength and the accelerating voltage of an electron was, in fact, shown by de Broglie to be

$$\lambda = h/mv = 12{\cdot}2/V,$$

where V is the accelerating voltage, so that it may be calculated that for an electron accelerated between two plates whose voltages differ by 60,000 volts (whose energy therefore is 60,000 electron volts) the corresponding wavelength would be 0·05 Å. The resolution for such a wavelength is therefore extremely high even for very imperfect lenses of very low numerical aperture; thus for an aperture of 0·001 the resolving power would be 300 Å. and for 0·01, 30 Å. The discovery that cylindrically symmetrical magnetic or electric fields can be used as "lenses" for electrons was therefore of the utmost possible significance.

The general construction of an electron microscope is very similar to that of the light microscope and we need to notice only a few major differences. Firstly, since the object has to be inserted in the (very high) vacuum system of the microscope then it must be dried very thoroughly. Secondly, since the object has to be transparent to electrons it must be very thin, thicknesses of more than 0·2 μ seldom being permissible. Extreme thinness in the object is necessary also to avoid the excessive overheating which would result from electron absorption. Thirdly, the image cannot be seen directly. The image is therefore thrown upon a fluorescent screen for viewing and is subsequently photographed, both the screen and the plate being, of course, within the vacuum system. Lastly, the magnification obtainable in this microscope (50,000 to 100,000) takes us down to an order of size hitherto unexplored, in which the detail depends on opacity to electrons and not to visible radiation.

On both accounts, therefore, interpretation of features of biological interest needs the greatest possible care.

Among the substances used by the pioneers of this instrument as objects of interest cellulose, too often in the form of dissected filter paper, was frequently used and innumerable photographs are on record. Unfortunately such dissected fragments produced little of interest in the botanical sense. The early work suffered, naturally enough, from a spate of enthusiasm without the experience necessary to ask, as it were, the right question in the right way. Recently, however, two investigations have been reported which seem to lead toward a clarification of the micellar aggregate position(41(*a*) and (*b*)). In the first of these to appear, Preston *et al.*, realizing that an experimental object was needed of which the structure was already known in some detail, and such that the treatment necessary would not lead to any distortion of structure, used the wall of the large vesicles of the marine alga *Valonia ventricosa* (see p. 92). Pieces of wall (which could be several millimetres across) were fixed to a glass slide and dried in a desiccator. The wall is, of course, much too thick to be examined directly and so a surface replica was made for observation in the microscope. Following a fairly recent advance in technique, the material was first "shadowed" with chromium. This is done by placing the glass slide, with the wall attached, into a high vacuum in a vessel which contains also a heated filament carrying a bead of metallic chromium. The mutual arrangement of the chromium and the slide is such that the atoms of the metal strike the surface of the slide at a small angle. In this way, any contours on the specimen are accentuated; elevations in the surface (Fig. 33) become covered heavily with particles of metal on the side nearer the filament, whereas the surface beyond is free of deposit. After the deposit has reached a suitable thickness the slide is removed and sufficient formvar or collodion solution poured over it that, when dried, the film can readily be removed from the specimen and yet be thin enough to allow the passage of electrons. The film takes with it the metallic deposit, so that the replica carries a faithful copy of the surface plus the metal.* It should be noticed that positions of thick metal deposits are opaque to electrons and therefore register in the photograph as white areas; places with no metallic deposit, *i.e.* the shadows, are registered as dark areas, and the reproduction of the specimen illustrated in the Frontispiece is such that this condition is maintained.

Detailed consideration of this beautiful photograph will be postponed

* It is now clear, however, that the specimen considered here is not a true surface replica; it contains at least two lamellae stripped from the wall.

to a later chapter; for the moment the chief point of interest lies in the striking demonstration, in a wall as nearly normal as a wall can be when looked at in this way, of the presence of extremely fine threads. The scale drawn on the photograph will show that these threads are about 250–300 Å. wide and are astonishingly uniform. We might have expected that the preliminary drying of the specimen should have caused the development of cracks in the wall but it seems highly probable that these would have been much more irregular than the structures we see here. It seems at the moment certain that these

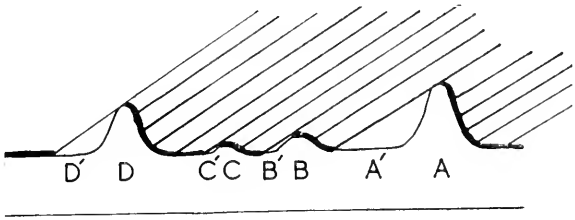

FIG. 33. Cross-section of the contoured surface of a specimen to show the effect of metal shadowing. The sides *A*, *B*, *C*, *D* of elevations toward the filament become coated with metal, but the elevations protect the far sides, *A′*, *B′*, *C′* and *D′* from metallic deposits. In the microscope, the parts *A*, *B*, *C*, and *D* are therefore opaque to electrons and *A′*, *B′*, *C′*, and *D′* are transparent. The angle of shadowing is exaggerated for clearness of figure.

minute threads are a feature of the cellulose in this particular wall. This refers, of course, only to the very surface of the wall, but in other photographs it has sometimes happened that the stripping of the replica has removed layers from the wall itself, and these layers, lying more deeply in the wall, show exactly the same structure. This latter type of observation constituted an accidental example of the spectacular advance in electron microscopy already made by Reed and Rudall (41(*c*)) using layer dissection to work out the detailed structure in the earthworm cuticle and underlying tissues.

More recent work, however, has shown that these microfibrils are not so uniform as the earlier photographs suggested (42(*b*) and (*d*)). Material teased apart, often after preliminary treatment with sulphuric acid at concentrations known to have no effect on microfibril diameter, shows the presence of microfibrils ranging in diameter from about 100 Å. up to about 450 Å. They are now known to be flattish ribbons rather than cylindrical rods (42(*d*)), a point to be discussed in more detail later on when the other Algae showing the same type of structure are discussed. The point has been made by Hodge and Wardrop (41(*a*)) for wood cellulose and by Ranby (74) for wood and cotton cellulose that the

fibrils there tend to range around 100–150 Å. in diameter. Frey-Wyssling and Mühlethaler (41(*b*)), however, find that celluloses from many sources including the tunicates contain microfibrils about 250 Å. in diameter.

As remarked elsewhere (p. 81) Ranby finds further that the microfibrils of cotton can be degenerated in sulphuric acid to give particles some 600–1000 Å. long and 50–100 Å. wide. The same is true of *Valonia* cellulose, though the conditions required for breakdown are much more severe than they are in cotton. It seems, however, a little uncertain whether these "micelles" are pre-existent in the microfibrils in any real sense. The fact, for instance, that microfibrils of the same diameter are found in primary wall cellulose, where the X-ray diagram is blurred, and in *Valonia*, where the X-ray diagram is very sharp, makes it difficult to picture the relationship between "micelles" and microfibrils. The general appearance of the wall seen in this way recalls very strongly the structure suggested by Meyer and illustrated in Fig. 25, though, of course, on a much higher scale of magnitude, and the photographs presented by the second group of workers of a variety of other cell walls present substantially the same picture.* Such photographs therefore leave open the possibility that the bulk of the cellulose chains, even in the non-crystalline regions, still lie roughly parallel to each other, since there is no suggestion of a "woolliness" at the edges of the threads which randomly oriented chains would tend to form. The broadening of the arcs in X-ray diagrams of cellulose would then be due to lattice defects and not to particle size. The question of the relation between "micelles" and microfibrils has been the subject of a recent discussion (76).

Lastly, we may perhaps notice that these photographs are in the most complete disagreement with the conclusions reached by Farr and her co-workers that cellulose is composed of spherical or slightly ellipsoidal particles visible under the light microscope. The threads observable in these electron micrographs are demonstrably longer, probably very much longer, than 10 μ and are therefore longer than the hypothetical particles are wide by a factor of about 10 at least.

* See, however, footnote, p. 88.

CHAPTER VI

Wall Structure in Thick Cell Walls. The Green Algae

Introduction

We now turn to the main purpose of this book—a consideration of the wall organization in plant cells in terms of the submicroscopic structure of cellulose. Whichever of the two aspects are temporarily adopted from which wall studies may be regarded (p. 2), and even if interest is confined to commercial matters, the information required is precisely the same. Perhaps we may note at the outset what these desiderata may be. They may be listed as follows:

(1) The molecular chains of cellulose usually lie along some particular direction in the cell. It is necessary to define this direction in terms of some recognizable morphological axis of the cell. Different layers in a wall often have different directions, when it becomes necessary to define the direction in each and every layer.

(2) Consideration can then be given to the run of the cellulose chains over the whole cell.

(3) With one possible exception (p. 81) cellulose is organized into micelles in the sense used here, and it is the average run of the micelles which is involved in (1) above. Since the micelles never do lie parallel to each other in any strict sense it is desirable, sometimes imperative, to know something about the "scatter" of the individual micelles about the common "preferred" direction, *i.e.* to know the *angular dispersion.*

(4) "Micelle breadth" can, and does vary widely and this may have a very considerable effect on the properties of a wall. It is desirable therefore to have some estimate of micelle dimensions. In view of the statements made earlier it should be clear that nothing can be said about micelle length; a more fundamental determination would be that of chain length, but the chains found in untreated walls are so long that variations such as occur are not expected to have any appreciable effect.

(5) Similarly the intermicellar "spaces" may occasionally diverge widely from the normal and in these cases it is desirable to have some measure of this divergence.

(6) Finally, and associated closely with (4) and (5), it is profitable to

know something about the relative amounts of the cellulose which is in the crystalline ("micellar") form.

It is not, of course, always easy, or even possible to make all these determinations, and attention will often be confined to (1) and (2). This is perhaps more particularly the case when investigating specifically the inter-relationships of wall structure and growth processes. Even here, however, observations of angular dispersion and micelle size also yield information of very considerable value.

Although chronologically the elongated cells of the higher plant have priority, it is in many ways more convenient to begin our survey of wall structure with the algae. As we have seen already, one algal cell—that of *Valonia*—was in fact used early in the development of modern ideas on cellulose structure, but it was not until 1937 that any serious attempt was made to investigate the algae for their own sake and for the light they may throw on problems arising in the higher plants. The advantages of studying the algae first are obvious. Here we have a wide variety of cell types, ranging from the unicellular forms with almost spherical cells, like *Protococcus*, through simple filamentous forms like *Spirogyra* and *Chaetomorpha* and branched filaments as in *Cladophora*, to the more complicated structures found in, for example, *Codium*. Many of the species available must, however, be ruled out here since cellulose is not present in the walls; we shall concentrate only on those which do carry this skeletal substance.

All these cells grow with little, if any, interference from neighbouring cells, and studies of the interconnection between growth and wall organization can be made here without the complexities involved with cells growing in tissues. This in the main forms the reason why the algae will prove of importance; not that they are without interest of themselves. Far from this, for they are a group of plants with extraordinary fascination, with a beauty of structure, even as revealed up to date, equal at least to any found in higher plants; and with intrinsic problems at least as interesting. It is clear that unless the growth forms in algae can be explained, studies of higher plants rest on a very infirm foundation. Since the group includes the largest single cell known, and this has been studied in great detail, it will be as well to consider this first.

Valonia

This alga is a native of the warmer seas and three species have been studied more or less intensively—*V. ventricosa*, *V. macrophysa* and *V. utricularis* (Fig. 34). Although these look rather different at a casual

glance, they have fundamentally the same type of cellular organization. The cells are large with very firm cell walls, a thin lining of protoplasm and a large central vacuole. They differ from normal cells in higher plants not only in their very much greater size but also because the cytoplasm contains many nuclei—they are said to be *coenocytes*. In a strict sense, therefore, they are perhaps not to be called cells at all, though from the present point of view this is a matter of little importance. In *V. ventricosa*, which has been the more common object of

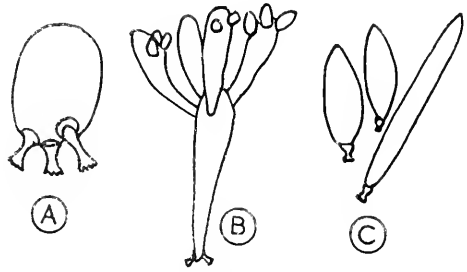

FIG. 34. Three species of *Valonia*.

(*a*) *V. ventricosa*. Large single vesicles with basal holdfasts.
(*b*) *V. utricularis*. Smaller vesicles; many attached laterally giving the impression of a palisade of cells.
(*c*) *V. macrophysa*. Vesicles, intermediate in size, with basal holdfasts (from which other vesicles occasionally grow out laterally).

investigation in this genus, the cells are very large indeed, ranging in size up to that of a pigeon's egg or even greater, and these giant cells occur singly. At the base of each cell, *i.e.* the end nearer the point of attachment to the rocky substrate, small pieces of protoplasm are cut off by lenticular walls and the outer, parent wall "blows out" into a cylindrical protuberance which serves to anchor the cell (Fig. 34A). There are several such *holdfasts* or *rhizoids* to each cell, but in this species they all occur close together at the base and no "watch-glass" cells are developed anywhere else over the cell surface. These small cells constitute the only signs of cellular differentiation found in this species. In the other two species the structures are essentially the same except that here watch-glass cells can develop anywhere. At the base these again produce holdfasts, but those situated farther away from this region produce new cells like the parent and these remain attached. This leads to the development of a coherent mass of cells each of which is, however, fundamentally solitary. In the main we shall deal here with *V. ventricosa* though it may be taken for granted that the descriptions refer also to the other two species.

The X-ray diagram

The first X-ray determinations of structure were made in 1930 by Sponsler during his determination of the unit cell of cellulose, but it was not until some years later that the particular organization of the cellulose in this plant was fully investigated (42(*a*)). Then it was found that if a *single* piece of cell wall is cut from a cell, allowed to dry flat after a preliminary washing with *N*/20 HCl to remove incrusting carbonates, and mounted in an X-ray spectrometer with the beam at right angles to the surface, a photograph such as that illustrated in Plate IV, Fig. 1, was obtained. Comparison of this photograph with that of wood cells (Plate I, Fig. 2) or hemp fibres (Plate II, Fig. 3) reveals immediately a very striking fact. In the photographs of the fibres there occur, on the equator, two arcs corresponding to planes of 3·9 Å. spacing and the line joining them (the equator, carrying also arcs corresponding to 5·4 and 6·1 Å.) is perpendicular to the direction of the cellulose chains in the specimen. In the *Valonia* photograph, on the other hand, *two* such rows of arcs occur (the arc corresponding to 6·1 Å. is missing, as mentioned earlier, p. 43), and discussed further below) so that the diagram from a *single piece* of wall is equivalent to that of two sets of ramie fibres crossed at about 90° to each other. In other words, the wall has not one but two sets of cellulose chains making an angle somewhat less than 90° with each other, and this opens up a problem of very considerable magnitude.

The first point to be solved concerns the spatial relation, within such a piece of wall, of the two sets of cellulose chains and here observation under the microscope gives a clue. Examination of a cross-section of the wall shows many superposed and rather distinct layers in the wall, and it might therefore be expected that the direction of the cellulose chains would alternate from layer to layer. This expectation is not, however, fulfilled. These layers can be stripped off individually from the wall and examined separately. Thus, if a piece of wall is dried on to a glass plate and a strip of sticky tape pressed on to it, then removal of the tape carries with it a thin layer of wall which can be washed off. *The thinnest layer which can be stripped in this way and can be mounted in an X-ray spectrometer still gives the crossed photograph typical of Valonia.* Each microscopically visible layer in the wall therefore is still heterogeneous in cellulose chain direction. This is shown to be true also by examination of cross-sections of the wall between crossed Nicols. If a section is cut at right angles to one set of cellulose chains, then we would expect, assuming that each layer did in fact possess

its own chain direction, that odd layers, say, would be bright (since the section is cut parallel to the chains and therefore shows high birefringence) whereas even layers (cut at right angles to the chains) should be dark. This is found not to be so; the layers are always uniformly bright. In some other algae, showing the same "crossed" structure, the regular alternation of bright and dark is, in fact, observed; but this is due to another effect altogether and we must be careful to remember the state of affairs in *Valonia* when we come to examine these other types.

Nevertheless it is certain that the two sets of cellulose chains must be segregated into layers even if these are submicroscopic; it is impossible to imagine the micellar structures as being interwoven like a fabric. Until the advent of the electron microscope and the shadow-casting technique, it was, however, remarkably difficult to prove this. One observation was made which seemed convincing, and it involves this principle. If the wall does consist of innumerable layers each with its own chain direction then, on mounting a piece of wall on a slide and examining it under a polarizing microscope, the optical conditions are somewhat as illustrated in Fig. 31 (p. 66) except that the angle between the major axes of the ellipses is more nearly a right angle. It follows, therefore, that the m.e.p. of the wall must lie in the acute angle between the two chain directions. This was found to be true, in this sense. Very few pieces of wall have in fact *one* m.e.p. More commonly, and as illustrated in Plate III, Fig. 4, the wall shows a "mosaic" of areas each with its own m.e.p., but each individual m.e.p. still lies within the acute angle between the cellulose chain directions. Since the chain direction is uniform over a few millimetres of wall, a moment's reflection will show that this fluctuation in the m.e.p. must mean that the relative *amounts* of the two sets of chains differ from point to point in the wall surface. It follows that the chains must be segregated into different layers. Further, it will be clear that, if we assume that segregation *is* into different layers, then from measurements of the phase difference shown by any small piece of wall, of the wall thickness and of the interstriation angle, the birefringence of each layer can be calculated (the method is too complicated to be discussed here; it can be found in the references quoted elsewhere (47(*e*)). The birefringence then calculated turns out to be about 0·06—the value for ramie fibres. This lends further support to the existence of such layers. Finally, the layers must be very thin since the *Valonia* wall shows complete extinction.

We can therefore picture the wall provisionally as built up of very many layers such that even layers, say, have chains lying in one

direction and odd layers in the other. Examination under the electron microscope gives a triumphant verification of this conception. A photograph of the outer surface of the *Valonia* wall obtained as described earlier (p. 88) is presented in the Frontispiece. This has been discussed already to some extent, but some other features may now be noticed. Firstly, the appearance of crossed threads is exactly what we should have expected in view of the X-ray photograph. Secondly, remembering that this is a *surface* then it is clear that the threads are segregated into layers which are extraordinarily thin. If we assume that the threads are circular in section then it seems certain that each layer is about as thick as the threads are wide, *i.e.* about 250–300 Å. Taking the thickness of the *Valonia* wall as 0·04 mm. ($=40\mu$ =400,000 Å.) and assuming the wall to contain 50% cellulose,* then this would imply the presence of some 700–800 layers. It has now been found possible to strip off these layers individually. Each has then only one set of microfibrils (42(*b*)). The segregation of the microfibrils of different orientation into separate layers is therefore placed beyond doubt.

It is of importance here to recall another feature of structure in *Valonia* already mentioned briefly (p. 43) and illustrated (Fig. 18). When a beam of X-rays is passed through the *Valonia* wall normal to its surface then no reflection is observed corresponding to planes spaced 6·1 Å. apart; these reflect most strongly when the beam lies *parallel* to the wall surface. This means that the 6·1 Å. planes tend to lie *parallel* to the wall surface. There is not complete restriction to this parallel position—in fact photographs taken with the X-ray beam running along the chains show that there is considerable angular dispersion amounting to about 70° on each side of the parallel position. Nevertheless the bulk of the planes are more or less parallel to the surface, and there are none lying at right angles to it. It is not clear if the chains as just laid down by the cytoplasm are arranged strictly with the planes corresponding to 6·1 Å. in the surface, this perfect arrangement being disturbed by secondary effects. The fact that these planes carry the densest array of —OHs is, however, most suggestive; for it may well be that, since the chains are laid down presumably at a hydrated protein interface, then the —OH groups would be held in the interface. This is most significant in view of the electron micrograph, for not only are the threads visible there arranged with beautiful parallel regularity but also must present the same "face" to the surface. Perhaps an analogy will help to clarify the position. If we threw down on a table a number of

* The cellulose content is not known; but the evidence obtained for the writer by P. H. Hermans of Utrecht, Holland, shows the presence of a large proportion of non-cellulosic substances.

pencils of circular section, then they would lie quite irregularly; they would point in any direction and the line of lettering in any individual might or might not be visible. We could then rearrange them so that they lay quite parallel to each other and with all the lines of lettering visible. This would be a very special arrangement; but it is precisely this condition which is manifested in *Valonia*. If the pencils were, however, elliptical in section (as in some pencils used by carpenters) with the lines of lettering on the broader face, then the lettering would always lie either on the upper or the lower side; correspondingly, if the threads visible in the electron microscope are in fact flat ribbons then the regular arrangement of the 6·1 Å. planes could receive ready and obvious explanation. It is now known (p. 89) that the microfibrils are indeed flat ribbons, the width/thickness ratio averaging about 2 but ranging up to 7.

Striation direction and chain orientation

In passing, it should perhaps be mentioned that the availability of comparatively enormous pieces of cell wall whose chain directions can thus unequivocally be defined made it possible to test the idea, already widely held though supported only by qualitative evidence, that the markings on cell walls known as *striations* reflect the directions of the underlying cellulose framework. In *Valonia* two sets of striations can be observed, though the ease with which they can be seen varies considerably even over small areas of wall, and this in itself makes it highly probable that striation direction and cellulose chain direction are one and the same thing. A quantitative test has, however, been carried out in this way. A piece of wall was mounted over a hole in aluminium foil, and a fine hair was cemented to it so that the hair lay parallel to one set of striations. This was then mounted over the slit of an X-ray spectrometer and a copper wire placed over the photographic plate was set parallel to the hair. This gave a reference line (the hair) on the specimen and a parallel one (the shadow of the wire) on the plate. It was then a comparatively easy matter to make the necessary observations, and it was found that striation direction and chain direction did coincide within the limits of observational error (42*a*).

This is, in many ways, particularly fortunate. If we can carry over this correspondence to other cells, and especially the minute cells of the higher plants, then it provides a ready method of determining cellulose chain direction even in the smallest pieces of wall provided these show striations. It is not, of course, self-evident that this correspondence will always obtain, but a similar correspondence has been found, for

instance, in conifer tracheids where we shall find it very useful. Care has, in any case, to be taken to distinguish between striations proper and the other wall markings with which these can be confused.

The organization of the wall as a whole

Thus far we have been dealing with small pieces of wall as experimental material and concentrating our attention on the detailed features of isolated fragments. It is now a fascinating problem to try to stick these pieces together again to see how the cellulose chains run in the

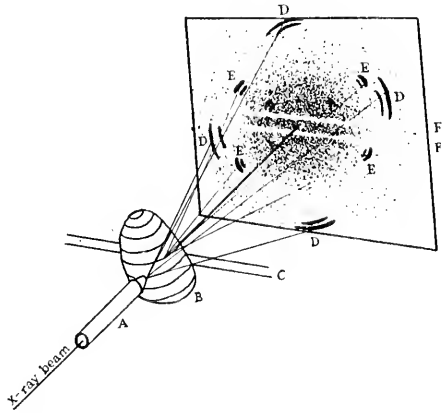

FIG. 35. Investigation of a whole *Valonia* cell. The empty dry cell *B* is held against the camera slit *A* by the two fine wires *C*. These are arranged parallel to those parts of one of the circles on the cell nearest the point being photographed. They cast shadows *FF* on the plate and therefore record the direction of the circle.

whole cell. The problem could, of course, be solved in exactly this "jigsaw" way. A whole cell could be cut up into pieces of some regular shape (one such way would be to cut out sectors in the same way as one peels an orange) and the striation directions could be observed under a microscope. These could then be plotted on the surface of a model of the original cell. In principle this would, in fact, be the quickest way but the chances of error are so enormous that it seemed undesirable to make any such attempt. Instead a much more laborious, but much more sure, X-ray method was adopted. The labour involved can be imagined when it is noted that each single photograph required an exposure time of five hours and, taking three photographs each day, the investigation still necessitated three years of uninterrupted work! The method used was as follows, and as illustrated in Fig. 35.

A cell of fairly regular shape was emptied through a fine capillary

tube inserted through the wall, washed out several times with distilled water, inflated with air and dried. A series of circles was then marked on the cell by mounting it on a rotatable spindle and holding a stylus against the wall surface while the cell was rotated. The circles were duplicated on a model of the cell some ten times the size of the cell itself. Now the cell was mounted in the X-ray spectrometer so that any point of its surface could be brought into contact with the slit and be held there by a system carrying two parallel wires; these could be arranged parallel to the tangent to the circles at the point of contact with the slit. In this way a photograph of the piece of wall touching the slit carried also the shadows of the wires which were parallel to the circles. The directions of the cellulose chains could thus be marked both on the specimen and on the model. Now observation of the striations had already shown them to be sensibly straight over at least 2 mm. Hence one of the cellulose chain directions was continued over a distance of $1\frac{1}{2}$ mm. and another photograph obtained. Repetition of this operation enabled a complete survey of one set of chains to be made over the whole wall surface.

The first set of chains to be followed appeared to make a great circle round the cell, passing over the base of the cell to the tip and back to the base. In some regions, and particularly at the base and tip, the photograph was very diffuse and it was, in fact, difficult to be sure that the chains did run back through the initial starting point—whether we had traversed a single great circle or only part of a flat spiral. Returning to the starting point, therefore, the second set was followed. Here the path was most certainly spiral, and the spiral was laboriously followed round and round the cell for nearly three years. As the spiral closed in more and more towards the base, the photograph became more and more diffuse and it became progressively clearer that a point was being approached at which the typical "crossed" photograph would not be obtained. This point was at last reached (Plate IV, Fig. 2). The model thus finally presented the appearance shown in Plate IV, Fig. 3. Clearly one set of chains forms a slow left-hand spiral round the cell which closes in at the tip and the base. The other set equally obviously forms a series of meridians, uniting the two "poles" of the spiral. It is clear, therefore, why the photograph becomes diffuse near these poles and why, at the poles themselves, it shows a series of circles instead of the usual crossed lines of arcs.

In view of what we shall find later in other algae, it is interesting to notice that the spiral set of chains in the *Valonia* wall follows the path of the so-called equiangular spiral. The correspondence is not

mathematically exact since, among other things, the shape of the cell cannot be defined with precision. Roughly speaking, however, the spiral is formed in such a way that it makes a constant angle with the meridians at each point of the wall.

In *Valonia* therefore, we have now a remarkably clear picture of the structure in the wall. The most important problem of all, however, and one which strikes deep at the roots of life itself, still remains unsolved. This problem will turn up again and again and under conditions where an attack seems more feasible, but this first example cannot be allowed to pass without some mention being made of it. At each point of the wall here, and over the whole surface, the submicroscopic layers alternate regularly in chain direction. As the wall is being deposited, therefore, and after one such layer has been laid down, there must be a sudden "switch" in some condition which involves the laying down of further material with the chains oriented in a direction nearly at right angles to that in the former layer. When this layer is completed, the process is repeated; but now comes the crux of the whole matter. In the third layer the chains, instead of being laid down in *any* direction, are laid *parallel to those in the last layer but one.* Now once a set of chains has been laid down it is quite conceivable that, unless something catastrophic happens, chains will continue to be laid down in the same direction by a sort of crystallization process. It is impossible to conceive, however, of an orienting effect of one layer through another in which the orientation is different. The conclusion seems inescapable that the mechanism responsible for orientation resides, not in the wall, but in the cytoplasm and probably at the very surface of the cytoplasm which is in contact with the wall. This is, of course, self-evident when a new cell wall is laid down either at a division or over the surface of a naked egg. Here we see that, even when a well-oriented wall is present, the orienting mechanism in the cytoplasm still takes precedence.

The problem to be solved has therefore at least two aspects. We may inquire, firstly, what changes either in the cytoplasm or in the surroundings, or both, cause the chain direction in the wall to be changed; and, secondly, why it is that alternate layers nevertheless have the same direction, a direction maintained over some 400 such "switches". These can be solved only through careful observations on material cultured in the laboratory (since the observational apparatus is too cumbersome to be carried to a remote sea coast). Unfortunately *Valonia* is difficult to keep in culture for long periods and until this is done, use must be made of other algae which show the same phenomenon and which can be collected regularly from less remote habitats. In some ways these

are less satisfactory, and even now only the first steps have been taken in an approach to the problem. These will be considered in the next section.

It is interesting from this point of view to note, however, the peculiar appearance in the electron microscope, of the innermost lamella of *Valonia*. This is the lamella which has just been deposited and is still in contact with the cytoplasm of the cell. It presents roughly the same appearance both in formalin-preserved (42(*b*)) and in freeze-dried (42(*c*)) material. The wall is covered by an amorphous mass pierced with holes of various diameter and this can be interpreted as the cytoplasm. Through the holes, or thinner places, the underlying fibrils are seen to be arranged at random, in marked contrast to the beautiful regularity of the lamellae deeper in the wall. The most interesting feature, however, is this. When this cytoplasm is swept away, isolated patches still remain firmly attached to the wall. These are most clearly in intimate association with the microfibrils; and one interpretation would therefore be that they are "islands" of cellulose synthesis. This would be in harmony with modern trends in biochemistry, for the need for islands of synthesis in the cytoplasm is turning up again and again. Such observations do not as yet do anything more than make the problem of microfibril orientation still more obscure. It does seem, however, that the microfibrils are produced first and are then oriented afterwards.

The filamentous algae

One general point does apparently emerge from the observations made thus far on *Valonia* in comparison with the condition already found in fibres. These latter will be discussed in more detail later on (p. 113 *et seq.*). At the moment let us put side by side the facts that in fibrous cells the bulk, at any rate, of the cellulose chains lie almost parallel to cell length, whereas in the bulbous *Valonia* there are two sets of chains running almost at right angles to each other. Then it is immediately clear that the walls of fibrous cells are much stronger parallel to their length than at right angles to it while in the walls of the alga the anistropy must be much less marked. Is this why *Valonia* cells are balloon-shaped while fibres are long and thin? This is a question often put by the uninitiated and we should perhaps pause a moment to consider its implications. Notice that the question involves almost a *non sequitur*. The *Valonia* cell is growing *while clothed in a wall exactly as described here*; the fibrous wall we have looked at so far is the secondary wall *laid down after change in cell dimensions had ceased*. The comparison is therefore invalid and discussion must wait until

data on the growing wall of fibrous cells is at hand. It will be seen then that, if the question is asked in the right way, there *is* some connection between growth and wall structure.

It is from this point of view that the filamentous algae are of especial interest. As far as shape goes they form a bridge between forms like *Valonia* and the cells of the higher plant, and they occur in such a variety of species that it is an exciting business to see whether there is any common underlying factor in the wall corresponding to the filamentous habit. The results of such determinations as have been made up to date cannot in any sense be regarded as final, but so far as can be seen at the moment the filamentous algae fall into three main groups. Only two of these will be discussed here; consideration of the third must be postponed until the X-ray diagram can be interpreted! Attention may therefore best be given first to those forms which most resemble *Valonia*.

GROUP 1. *Cladophora, Chaetomorpha, Rhizoclonium, etc.*

These forms are natives of the temperate zone and therefore occur, in greater or less abundance, around the coast of Britain as well as in its fresh waters. *Cladophora* is typified as a much branched, multicellular filament (Fig. 38), each cell containing many nuclei. One species, *Cl. prolifera*, is much larger than the others, and this was the first to be studied. In all species the branches occur as a bulging of a cell at the end nearer the filament tip (Fig. 36(*a*)) giving the appearance of being "blown" out from the parent cell. Growth occurs exclusively in the upper part of the apical cell, causing cell elongation which is followed regularly by transverse division into two cells. The sub-terminal cell is capable of limited growth in that the half of it nearer the filament tip continues to extend for some little time after it has been cut off from the terminal cell. The branch cells grow in a similar way through the agency of a tip cell, so that the form of the plant becomes somewhat complicated. In one species, *Cl. gracilis*, the branches along any one filament occur first on one side, then on the other, with some regularity. The whole plant may be up to three or four inches in length. The organization of the other two genera, *Chaetomorpha* and *Rhizoclonium*, is somewhat similar, except that the filaments are usually unbranched and growth is not confined to the tip cells.

With one exception, it is impossible in these species therefore to investigate single cells in an ordinary X-ray spectrometer, and bundles of parallel cells must be used. This introduces difficulties in the case of *Cladophora* but even here, with care, it is possible to arrange a sufficient

number of the filaments parallel to each other so that the diagram of those branches which are not parallel (and whose diagram is therefore spread over a circle) does not interfere. The X-ray diagrams of mature specimens of the three species are almost identical and a typical

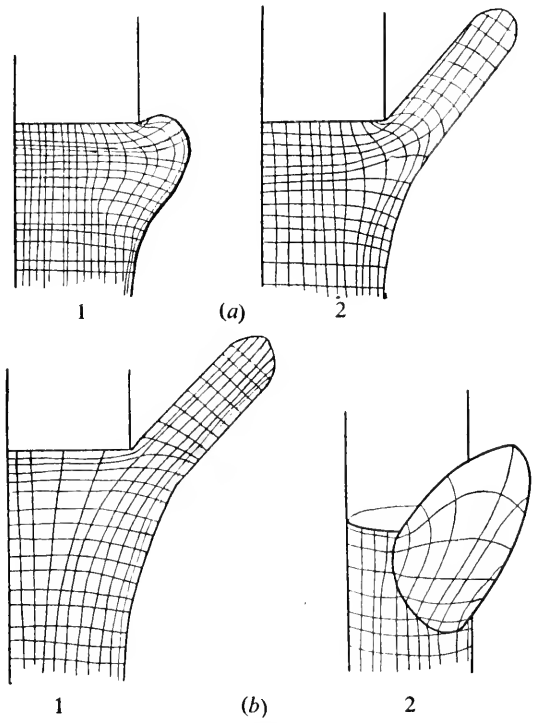

FIG. 36. The development of a branch in a filament of *Cladophora*. Part of the wall at the end nearer the filament tip becomes "blown out" into a protuberance which then continues to grow like the apex of the parent filament.

(*a*) The run of the striations on the outer wall lamellae at a branch (1) young stage, (2) older stage.
(*b*) Two views of the run of striations in inner lamellae, deposited after formation of the branch.

The striations in (*a*) (1) suggest that the branch arises by a lateral "blowing out" of the wall. Layers deposited later are so adjusted as to give a smooth run of striations from the parent cell to the branch.

example, obtained as usual with the beam normal to the length of the filament, is given in Plate IV, Fig. 4. In order to achieve a clear interpretation of this diagram, which will prove of material importance later on, it will be as well to turn first to the species *Cl. prolifera*, whose cells are large enough to be examined individually. When one of these cells

is cut open, laid out flat and dried, then the diagram resulting with the X-ray beam normal to the surface is indistinguishable from that of *Valonia* (Plate IV, Fig. 1). The wall of this species also, therefore, consists of two sets of cellulose chains crossing each other almost at right angles. Here again, too, two sets of striations are visible on the wall—and this is true of most species—which correspond in direction

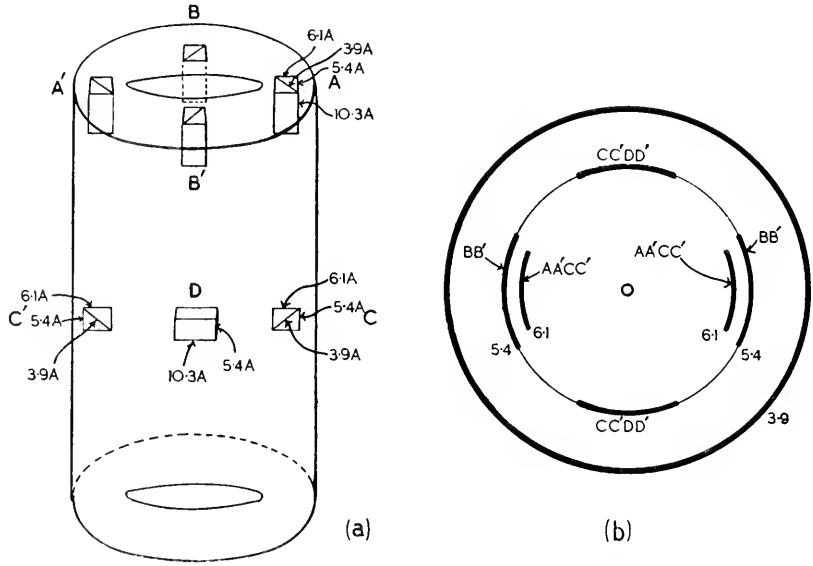

FIG. 37(*a*) The mutual orientation of the unit cells of cellulose corresponding to the directions of cellulose chains in cells towards the apex of *Cladophora*.

Upper half, arrangement corresponding to longitudinal chains.
Lower half, arrangement corresponding to transverse chains.

FIG. 37(*b*). Diagrammatic representation of the corresponding X-ray photograph, with the beam perpendicular to cell length. The lettering of the 6·1 and 5·4 Å arcs denotes the positions in Fig. 37(*a*) from which the arcs are derived. Note that the equatorial 5·4 Å. arc arises from longitudinal chains and the meridional arc from the transverse, while the equatorial 6·1 arc is "mixed".

to those of the cellulose chains. It seems therefore very reasonable to assume that the organization of the wall is very similar to that obtaining in *Valonia*. Towards the tip of the filaments of mature plants in fact, the striations, of which more will be said later, run one set in a slow spiral and the other in a very steep spiral, *i.e.* almost parallel and perpendicular to cell length. The organization of a whole cell of the filament (ignoring the end walls) must therefore be somewhat as in Fig. 37(*a*). This can be checked against the X-ray diagram, Plate IV, Fig. 4 and Fig. 37(*b*). Taking the arcs corresponding to planes of 6·1 Å.

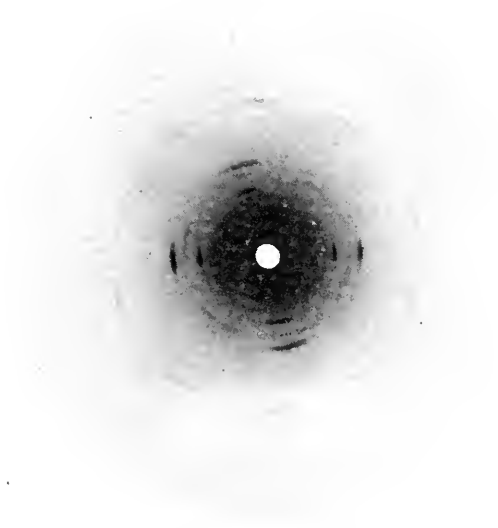

FIG. 1. Typical X-ray diagram of a single piece of *Valonia* wall, beam perpendicular to the surface. CuK_α radiation.

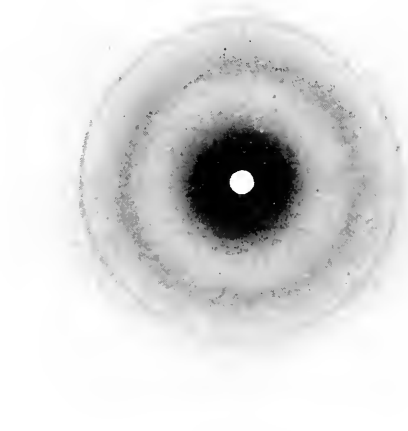

FIG. 2. X-ray diagram of the *Valonia* wall at a "pole" of its structure.

[*Facing p.* 104

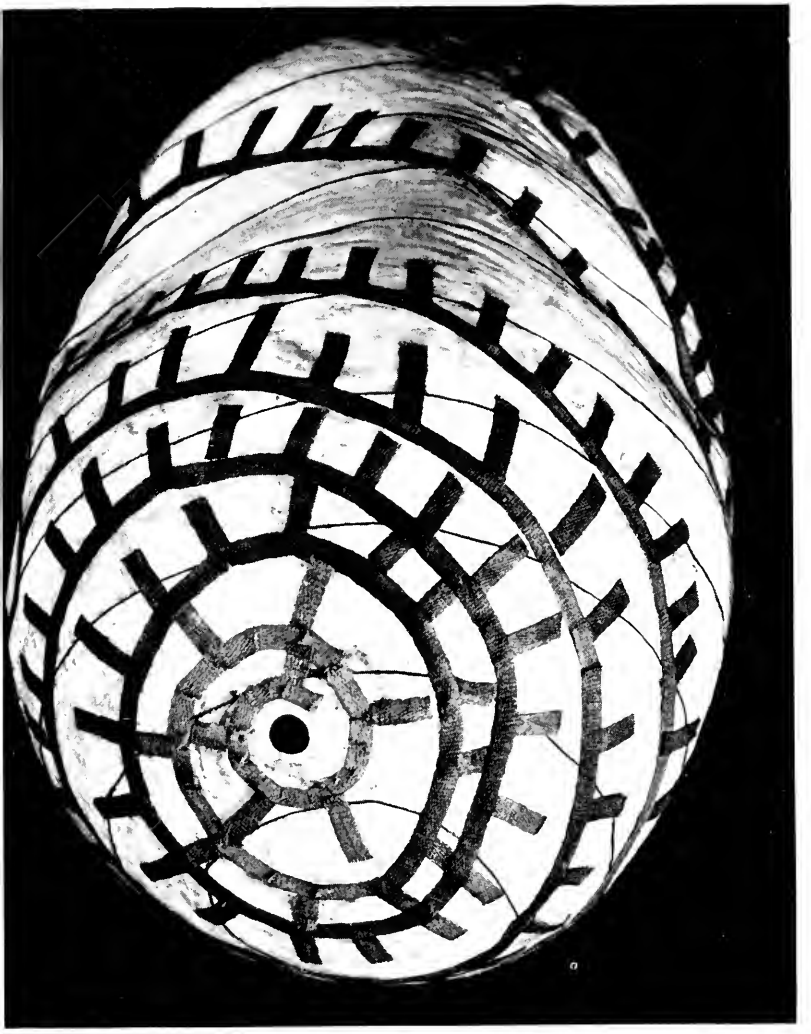

FIG. 3. Model representing the wall structure of a *Valonia* vesicle. The broad lines on the model represent chain directions. Note that one set of chain forms a slow spiral round the vesicle while the other clearly forms meridians radiating from the visible "pole" and closing in again on the basal "pole".

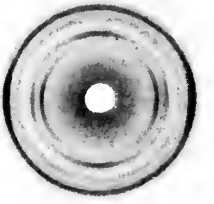

FIG. 4. X-ray diagram of a bundle of parallel filaments of *Cladophora* sp., beam perpendicular to filament length, CuK_α radiation. Filament length parallel to long edge of page.

spacing first, the arc on the equator obviously arises from both the longitudinal set of chains (positions *A*, *A'*) and the transverse set (positions *C*, *C'*). The equatorial 5·4 Å. arc, on the other hand, can arise only from the longitudinal set (positions *B*, *B'*), and the meridional arcs only from the transverse set (positions *D* and the one on the other side of the set and not shown). The 3·9 Å. reflections arise from both the longitudinal set (positions intermediate between *A* and *B*) and, diagonally, from the transverse set (positions *C*, *C'*) and are therefore multiple. The arrangement of the unit cells illustrated diagrammatically in Fig. 37(*a*), therefore, explains completely the diagram actually obtained and it may be concluded that all filaments of *Cladophora* presenting this diagram are wound with two sets of cellulose chains, and that the planes of 6·1 Å. spacing again tend to be parallel to the wall surface. This is fully supported by observation of striation directions, though naturally no complete check can here be made that striation direction and chain direction are quantitatively the same. Further, the walls observed in face view between crossed Nicols are uniformly almost dark, as a rule, as could be expected from this type of structure.

The position is somewhat different towards the base of a plant. Here both sets of chains form spirals round the cell the "steep" set being less steep than in the upper parts of the plant and the "flat" set more steep. A further peculiarity lies in the fact that, in any individual cell, the flatter spiral becomes flatter and the steeper spiral steeper towards the end of the cell nearer the tip cell (Fig. 38); and the flatter spiral at any point in a cell becomes flatter as we pass from the base of the filament towards the tip and at the same time the steep set becomes steeper. At any cross wall, therefore, there is a sudden change in striation direction which is less marked towards the apices of a filament. It should be particularly noted that, since these spirals are of opposite sign, then these changes are such as to maintain the angle between their windings constant throughout a filament.

It becomes a little difficult, therefore, to understand the very different morphology of *Valonia* and *Cladophora* in terms of changes in shape due to interaction of internal hydrostatic pressure and the elastic properties of the walls. In the basal regions it is understandable that, since both sets of chains constitute rather flat spirals, the cell will blow out into a cylinder rather than a sphere; but in the upper regions there seems to be no such direct connection between wall structure and growth form. It should particularly be noted that growth continues at the tip long after the steep set of striations has become longitudinal, and from this point on changes in cell dimensions involve no further change in chain

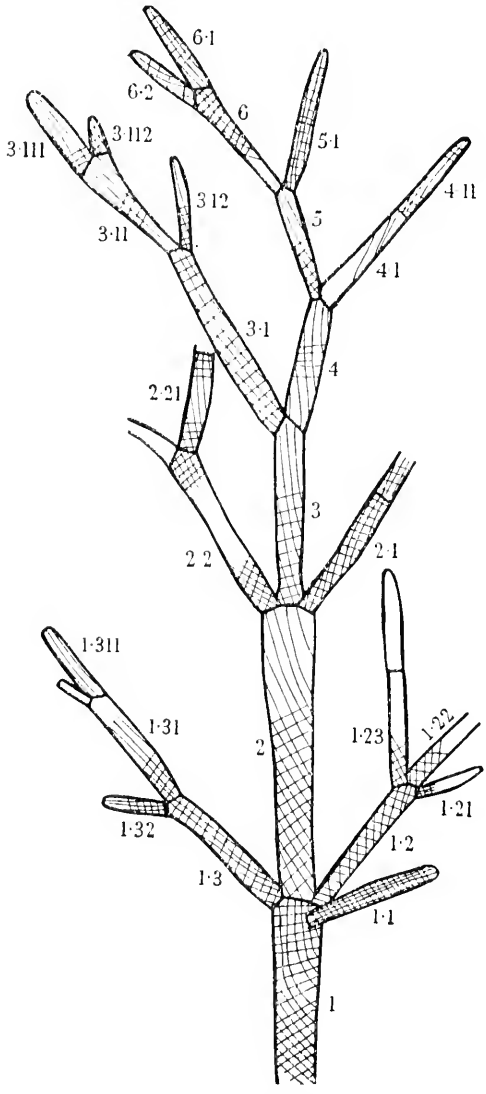

FIG. 38. Diagram of a branching filament of *Cladophora prolifera* to show the run of striations (and therefore of cellulose chains). One set of chains, or both, are omitted only in those regions where opaque cell contents made observation impossible.

orientation. Unless, in fact, there is some very considerable difference in wall structure between mature walls and that at the very tip of the terminal cell, it is difficult to see how the cell grows at all, and this applies also, of course, to *Valonia*. In general the same thing is true for

fresh-water *Cladophora* (43(*c*)) and for *Chaetomorpha*(43(*b*)) and for *Rhizoclonium*(43(*c*)),* where, however, the difficulties are still more acute since these filaments grow by intercalary growth so that the X-ray diagrams are, presumably, those of growing walls. The problems involved in the growth of these cells will be examined later.

Turning therefore from these difficult and, as yet, completely unsolved, problems, note may be taken of recent attempts to explore the complex of factors involved in the periodic "switch" in wall structure found first in *Valonia* and now in these filamentous forms. The first point of significance is illustrated in Table V. This is one sample of many similar determinations of striation direction, and it will be clear from inspection that the interstriation angle tends to be constant. The correlation coefficient between the two directions is in fact −0·59. The two directions are therefore not independent. In seeking a possible line of attack on this problem, we were encouraged by the observation reported by Anderson and Moore(44) that cotton hairs grown in constant light no longer show the wall lamellation so typical of normal cotton. Attempts have therefore been made to ascertain if here too the change in wall structure (of a much more fundamental nature than that observed in cotton, however) is associated with the change, in nature, from light to dark. All three filamentous forms under discussion here have been grown under constant light conditions and carefully compared with controls growing under the normal alternation of light and dark. These algae will not long tolerate exposure to temperatures much above 18°C., presumably on account of the gross bacterial infection which then occurs, but the fresh-water species have nevertheless been grown successfully under the two experimental conditions. Marine species are somewhat easier to deal with, and successful cultures have been maintained both in the laboratory at Leeds and, through the courtesy of Professor Hobson and Dr. Bull, at the Dove Marine Laboratory at Cullercoats. The latter site is remarkably good for this kind of work, for flowing sea water is continually available inside the laboratory.

The major difficulty in attempting interpretation of these observations lies in the fact that the algae concerned grow very slowly, so that even after some months under experimental conditions the bulk of the walls, in all cells except at the tips of filaments, were present before the experiment began; only a relatively thin innermost layer of wall has been laid down under the experimental conditions. Thus we expect, not a complete change in the X-ray diagram, but merely a modification;

* The same structure has now been found in *Siphonocladus* but, strangely enough, not in the *Spongomorpha* group of *Cladophora*.

TABLE V

The inclination, in degrees to the longitudinal axis, of striations in filaments of Cladophora prolifera (fig. 38)

A *negative sign indicates a left-hand spiral, the absence of sign a right-hand spiral.*
a *end of cell nearer tip of filament.*
b *centre of cell.*
c *end of cell farther from tip.*
Spaces left blank indicate that the striations are obscured.

Cell	*Inclination*						*Inter-striation angle*		
	a		b		c		a	b	c
1	0·0	89·5	−20·0	76·0	−33·5	60·5	89·5	96·5	93·5
1·1	—	—	0·0	74·0	0·0	—	—	74·0	—
1·2	− 9·5	76·5	−18·0	76·5	−23·0	76·5	86·0	94·5	99·5
1·21	—	—	—	—	− 4·0	85·0	—	—	98·0
1·22	—	—	—	—	−20·0	70·0	—	—	90·0
1·23	—	—	—	—	−11·5	75·5	—	—	87·0
1·3	− 5·0	79·0	− 4·0	86·0	−10·0	68·5	84·0	90·0	78·5
1·31	—	—	− 3·5	—	− 3·5	78·5	—	—	82·0
1·311	− 0·0	—	0·0	—	− 6·0	—	—	—	—
1·32	0·0	—	− 2·0	72·5	− 3·5	79·5	—	74·5	83·0
2	−10·5	—	−28·0	67·0	−15·0	56·0	—	95·0	80·0
2·1	0·0	83·5	−13·0	78·0	− 7·0	77·0	83·5	91·0	84·0
2·11	—	—	—	—	− 6·0	72·5	—	—	78·5
2·2	0·0	75·0	—	—	−21·5	67·0	75·0	—	88·5
2·21	−10·5	84·5	−12·5	—	−19·5	72·0	95·0	—	91·5
3	0·0	—	− 9·0	84·0	−18·0	73·0	—	93·0	91·0
3·1	− 3·0	81·0	−10·0	81·0	−11·0	80·0	84·0	91·0	91·0
3·11	− 8·5	—	− 4·0	74·5	− 3·0	—	—	78·5	—
3·111	0·0	—	0·0	—	8·0	98·0	—	—	90·0
3·112	− 9·0	72·5	− 9·0	72·5	− 9·0	72·5	81·5	81·5	81·5
3·12	− 2·5	—	− 7·5	84·0	− 8·5	85·0	—	91·5	93·5
4	− 6·5	—	−14·5	74·0	− 4·5	—	—	88·5	—
4·1	− 8·0	—	− 4·0	—	− 0·0	—	—	—	—
4·11	0·0	83·0	− 3·5	—	−10·5	73·0	83·0	—	83·5
5	− 7·0	—	−11·0	72·5	−10·0	79·0	—	83·5	89·0
5·1	0·0	87·0	0·0	85·5	− 8·0	76·0	87·0	85·5	84·0
6	0·0	90·0	−17·5	73·0	—	—	90·0	90·5	—
6·1	0·0	90·0	0·0	91·0	−11·0	—	90·0	91·0	—
6·2	0·0	78·0	− 5·0	72·0	−20·0	71·0	78·0	77·0	91·0

the chances of a complete change must wait until the very tips of the filaments can be studied in an X-ray microcamera. The results are nevertheless rather convincing(45). They may best be understood by reference to Fig. 37. It must be remembered that the meridional arc of 5·4 Å. spacing arises solely from the transverse set of chains, while the corresponding equatorial arc corresponds to the longitudinal set.

Assuming that the run of the chains is the same both in the continuous light series and the controls (and the run of the striations is, in fact, the same) then the ratio of the intensity of the meridional the equatorial arcs gives a relative measure of the proportion of the chains in the wall which are transverse. In the one case examined—marine *Cladophora*, both in the Leeds laboratory and at Cullercoats—this ratio is significantly higher in continuous light. Plate V, Fig. 1, gives a forceful illustration of this point. Two other types of observation further substantiate the resulting conclusion, that under constant light conditions more chains are laid down in the transverse direction than in the longitudinal. Thus, when cells grown under normal conditions are examined between crossed Nicols the (double, upper and lower) walls seen in face view are non-birefringent. In cells from filaments grown for some months in continuous light, however, the walls are strongly birefringent and the birefringence is negative. The non-birefringence in the former case is due to the presence of chains in approximately equal amounts in two directions almost at right angles. In the latter, therefore, the negative birefringence means that more transverse chains are present. Again, the morphology of cells grown in constant light is rather different (sometimes very different) from normal; numerous swellings appear in the otherwise cylindrical filaments, and this can only mean a disturbance in wall structure such as we have in mind now.

Complete confirmation of this point must await further results from cells which have grown *only* in continuous light, but at the moment the evidence does seem rather convincing. Nothing is known as yet concerning the way in which light is associated with this switch in wall structure. It seems rather clear that the periodic change in wall structure must imply a corresponding periodic change in the surface of the cytoplasm which is in contact with the wall and which is presumably instrumental in defining the orientation in the wall. This is a point we shall return to again.

GROUP 2. *Halicystis, Hydrodictyon, etc.*

The second group of algae which should come under notice here can in general be typified by *Halicystis* just as the last group was typified by *Valonia*. *Halicystis* resembles *Valonia* in consisting of very large, solitary cells which do not, however, reach the size of the latter. One might therefore expect *a priori* that the wall structure would be approximately the same. This, however, is not so; there is at least one very marked difference between this group and the last. Whereas the walls

dealt with up to now have consisted of so-called *native* cellulose, the walls in this group appear to consist of cellulose, it is true, but this has been reported to be in the form known as *hydrate* or *mercerized* (46(*a*)).

If native cellulose is dispersed and coagulated from solution, or is swollen in 18% caustic soda and washed, then a product is obtained which gives a new diffraction pattern. This product is naturally still cellulose and has the same analytical composition as the native form. It is, however, more reactive chemically, with a stronger affinity for dyes, etc., and the fact that its X-ray diffraction pattern is different from

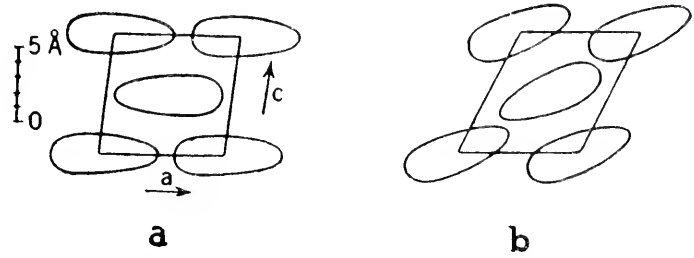

FIG. 39. Projection of the unit cell on the *ac* plane in (*a*) native cellulose (cellulose I) and (*b*) mercerized cellulose (cellulose II). (Reproduced from *Physics and Chemistry of Cellulose Fibres*, by P. H. Hermans, Elsevier, 1949, by permission of the author.)

that of the native form shows that it is different crystallographically. Just as the unit cell for the native form has been obtained so here, in the same way, a different unit cell has been calculated and a projection of this cell on the *ac* plane is given in Fig. 39(*b*) together with a similar projection of native cellulose (Fig. 39(*a*)) for comparison. This is usually regarded as the stable form of cellulose, the native form being metastable, chiefly on account of the ease with which native cellulose can be transformed into the mercerized form and the difficulty in reversing the process. It is therefore of the greatest interest that the alga under discussion here appears to have cellulose in the mercerized condition. The wall is closely like a sheet of cellophane, though there are small differences. At the time this important discovery (46(*a*)) was made with *Halicystis* it was not known whether or not this alga was unique in its wall composition. We now know, however, of a whole range of algae with precisely the same structure (46(*b*)).

The X-ray patterns have, as a matter of fact, at least three components. Mercerized cellulose has three characteristic arcs on the equator, corresponding to spacings 7·4 Å., 4·4 Å. and 4·0 Å. (equivalent to the 6·1 Å., 5·4 Å. and 3·9 Å. of native cellulose). All three arcs are

prominent in these diagrams. In addition, however, there is often an arc corresponding to planes spaced 12·5 Å. apart and oriented parallel to the 7·4 Å. planes. This possibly implies the presence of a second substance in crystalline form, and was interpreted in this way by Sisson. Since that time, however, long spacings of this kind have repeatedly been found even in native cellulose and it is not now clear just what such spacings mean. Superposed over the whole pattern there is a diffuse scattering indicating non-crystalline substances such as often appear, however, also in native celluloses. As regards the organization of the cellulose in the wall, mercerized cellulose of course is still composed of molecular chains, and these are again united into micelles in the same sense as used for native cellulose. Here, however, any resemblance to *Valonia* ceases. In diagrams obtained by passing an X-ray beam normal to the wall surface, a number of complete rings are observed (Plate V, Fig. 2) quite unlike the sharp arcs in *Valonia* and the 7·4 Å. arc is missing. This means that the molecular chains are oriented in the surface at random. If the beam is passed parallel to the wall surface, however, the 7·4 Å. arc is now very strong. The planes of this spacing are therefore parallel to the wall surface, just like the 6·1 Å. planes in *Valonia*; and again these are the planes richest in —OH groups. It seems therefore to be a general rule, in the algae at least, that planes rich in —OH groups tend to lie parallel to the wall surface.

In spite of the similarity in shape, therefore, and of the fairly close relationship, the cells of *Halicystis* and *Valonia* are very different. Nevertheless it will be clear that the architecture in *Halicystis* is still such that the wall is isotropic in its physical properties, so that a uniform pressure within the cell will cause the wall to stretch uniformly in all directions. If a piece of wall is stretched mechanically in one direction, then the molecular chains tend to align themselves in the direction of stretching, just as they do in the case of cellophane. During growth, however, the tension in the wall will be approximately the same in all directions and it would therefore be expected that the cell would remain spherical.

So far, so good. When the filamentous forms in this group are considered, however, difficulties immediately again appear. Problems of growth are never so easy as that!

Let us take as an example *Hydrodictyon*. Although this is not a typical filament, the constituent cells are rather long cylinders and are more easily handled than the smaller cells of the other species. The plant takes the form of a net of cells, each cell steadily growing larger as time goes on, and therefore making the net larger. Even at their

largest, however, the cells are too small to be examined singly by X-rays without the use of a microcamera. Bundles of them can, however, be pressed flat and photographed with the X-ray beam in any chosen direction to the flattened faces and therefore to the wall surface, and the resulting diagrams are exactly like those of *Halicystis*. The wall structure must therefore be similar. How, then, does it come about that these cells are cylindrical whereas those of *Halicystis* are spherical? Here we meet the same difficulty as in the first group of algae, and again must leave the matter until later. One point may, however, be noted here. As mentioned above, when a piece of wall is stretched, a reorientation of the micelles is observed. When *Hydrodictyon* is growing, on the other hand, no reorientation occurs although the cells increase in length much faster than in girth. Here again therefore we have the clearest evidence that growth in a cell does not cause a strain in the wall such as to cause a reorientation. Indeed, this particular evidence makes it very doubtful if the wall undergoes any passive strain at all. Either these cylindrical cells, which are attached at each end to similar cells, must grow by localized insertion of new wall material (comparable with the apical growth of *Cladophora*, for example) or, if the whole wall is involved uniformly in the process, then growth is not just a question of passive extension by internal pressure. These questions will be considered further in a later chapter.

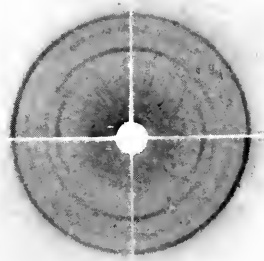

FIG. 1. Comparison of the X-ray diagrams of *Cladophora* cultured under constant illumination and under normal light-dark alternations. Photographic details as in Plate II, fig. 3.

Upper right, lower left: light-dark alternation.
Upper left, lower right: continuous illumination.

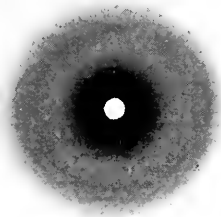

FIG. 2. X-ray diagram of a bundle of parallel cells of *Hydrodictyon*. Beam perpendicular to cell length, CuK_α radiation. Note (1) the presence of complete circular arcs demonstrates random orientation. (2) the inner circle corresponding to a spacing of about 7·4 Å., suggests clearly the presence of cellulose II instead of cellulose I.

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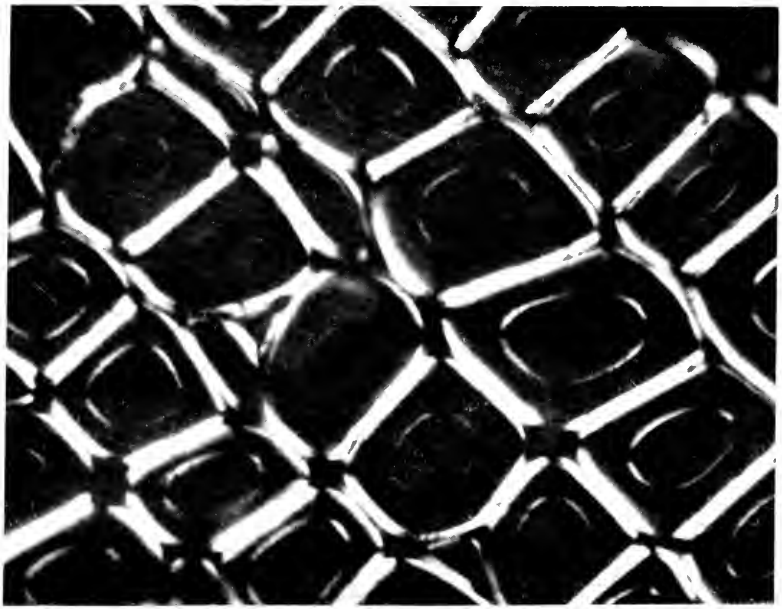

FIG. 3. Transverse section of tracheids in *Pinus radiata* between crossed Nicols. Note (1) a dark "middle lamella" (2) a narrow, bright, outer lamella in the secondary wall (3) a broad, dark, lamella within, and (4) often a very thin, bright, innermost lamella.

CHAPTER VII

Wall Structure in Thick Cell Walls. Flowering Plants

Layering in xylem cells

TURNING, THEN, to the more familiar types of plants it is found that, in spite of the very different form of the cells involved, and in spite of their very different method of development, the fine structure of the walls is in broad outline remarkably similar. We may perhaps turn our attention first to those features which are visible microscopically, and examine in the first place the *tracheids* which are supposed to be the primitive cell type, from forms like which the more specialized cells of the xylem have developed. Tracheids can be recognized in macerated material as elongated cells with tapering, but seldom pointed, ends with moderately thick cell walls and prominent pits. In transverse section it is sometimes difficult to distinguish these from fibres if the section has not included a pit, so that it will be as well to take up this particular aspect of the study with the wood of conifers since, apart from parenchyma cells and the ray tissue with which there can be no confusion, the wood is composed exclusively of tracheids.

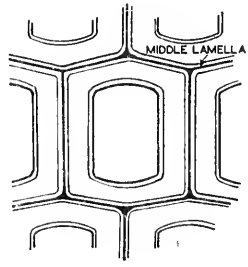

FIG. 40. Diagrammatic representation of a transverse section of a conifer tracheid in late wood, together with parts of the contiguous cells. The cells are held together by a middle lamella; the secondary wall is divided into three lamellae—the inner and outer ones narrow and the central one thicker. The primary wall is too thin to show on this scale.

If a thin transverse section of the wood of a conifer is examined under the microscope then it can be seen, particularly if the section has been stained in safranin or congo red, that the wall is layered (Fig. 40; Plate V, Fig. 3). Commonly, three such layers are visible in each wall (and therefore six in the double wall between each pair of cells) whose extent depends on the region of the wood from which the section is taken. In the thicker walled late wood produced towards the end of the growing season, the central layer is thick and the outer and inner layers comparatively thin. In the thinner walled early wood the central layer too is thin and, in fact, may be thinner than either of

the other two layers, so that the difference in wall thickness is to a large extent governed by the thickness of the central layer. This was first pointed out by I. W. Bailey. Layering of an exactly similar type is found in fibres, both in the xylem and the phloem, and in some vessels in Angiosperms.

This layering is brought out in a much more striking way when observed between crossed Nicols under a polarizing microscope (Plate V, Fig. 3). The outer and inner layers are invariably bright (the inner layer being often less bright than the outer), and the central layer is dark, or at least much less bright. The outer and inner layers, therefore, as seen in transverse section, are highly birefringent and the central layer is almost isotropic. This calls to mind very strongly the condition in the alga *Cladophora*, where a similar phenomenon was found to arise from layers alternately cellulose-rich and cellulose-poor. Further examination shows, however, that the two cases do not run parallel. When conifer wood is examined in thin longitudinal section the bulk at least of the wall is highly birefringent; it is difficult to be sure about the outer and inner layers but at any rate the central layer, which was isotropic in transverse section, is now birefringent. All three layers therefore contain abundant cellulose and explanation of the optical heterogeneity must be sought in some differences in the cellulose matrices themselves.

Looking back on the structural features of cellulose (Chapter V) and the relation of structure to birefringence (Chapter IV) it will be obvious that such a difference in birefringence in two neighbouring layers which must have very similar chemical make-up could arise from any one or more of the following:

(*a*) Since the "micelles" are optically equivalent to positive uniaxial crystals, then a variation in birefringence could arise from a variation in the orientation of the micelles. Here there are two possibilities.

(i) The change might be due to a difference in the net orientation of the micelles, and this would mean that the micelles in the outer and inner layers must lie more or less transversely in the cell forming, therefore, either transverse circles round the cell (if the micelles are quite transverse) or flat spirals.* The micelles of the central layer, on the other hand, must lie more or less longitudinally. This was the suggestion first made by Bailey and Kerr (47(*a*)), who called attention to the whole phenomenon. They further succeeded in producing strong corroborative evidence for this view. Thus, if isolated

* More correctly a helix. The term spiral is, however, so widely in use in the botanical literature that the term is retained here to avoid confusion.

cells or longitudinal sections are swollen, then striations can often be observed which run more or less transversely in the outer layer and more or less longitudinally in the central layer; iodine crystals grown in the wall take up two orientations, the needle-shaped crystals lying transversely in outer layers and more nearly longitudinally in central layers; and finally fungal attack develops cavities in the cellulose in these two directions(47(*b*)). Such evidence alone, however, is hardly convincing since in all these observations the wall has been strongly swollen, or subjected to other equally undesirable processes.

(ii) The change might be due, not to a net change in orientation of the micelles, but to a change in the angular dispersion while retaining the net preferred orientation. This was the suggestion made by the writer following largely the results of the X-ray analysis of tracheids and other cells showing similar optical heterogeneity. This type of dispersion change is one which undoubtedly does occur in these cells and is certainly necessary to explain some of the physical properties of the cells.

As will become clear later on, it is certain that both of these factors are operative. There are nevertheless other aspects of cellulose structure which could also be involved, about which nothing can at the moment be said with any certainty. These will be referred to again later; at the moment we may perhaps notice them in passing.

(*b*) The volume percentage of cellulose present might be sufficiently different to play a part in the optical heterogeneity. Such a connection is, however, difficult to establish until methods are devised for the isolation of the separate layers in quantities sufficient for at least microchemical analysis. The evidence which can at present be adduced is conflicting. On the one hand, macrochemical analysis of wood has shown that the percentage of cellulose is the same in both early and late wood and, since the central layer is well developed only in the latter, then it could be deduced that the composition of this layer cannot be far different from that in the narrower layers on each side of it. On the other hand Lange, using the method of infra-red microscopic analysis developed by Steenberg, has produced results which suggest that the outer layer at least has considerably less cellulose than the central layer. There remains, therefore, the distinct possibility that the percentage composition of the layers may be of importance.

(*c*) Finally, and even if the percentage composition turns out eventually to be not sufficiently different, it still may be that the percentage of crystalline cellulose is different. This is a possibility we can hardly begin to assess at this time, but there is even now some small evidence,

following the method of Hermans on results obtained by him from material supplied by the writer. This will be discussed later, however, after the X-ray diagram has been presented.

The m.e.p. of conifer tracheids

It is not feasible to decide between these possibilities, however, or to interpret the X-ray diagram even of such a simple tissue as conifer wood without looking a little further into the optical properties of tracheids. This is due, of course, not to any defect in the X-ray method, but as a consequence of our present disability, with occasional exceptions to which we shall refer again, to examine single tracheids by this means. In the exceptional cases optical and X-ray analysis yield results which are in complete harmony, so that there can be no doubt of the validity of carrying over observations made on single cells under the polarizing microscope to the interpretation of X-ray diagrams of wood sections. It should, of course, be remembered that the isolation of cells for examination under the microscope removes lignin and other incrusting substances, so that the material is presented for observation under slightly different conditions in the two methods. Fortunately this seldom produces any undesirable complicating features.

In order to determine the m.e.p. it is necessary first to devise some way in which single walls of the cells can be made available for observation. This has, in fact, been done in a way which seems remarkably simple when we remember that the cells concerned are of the order of 20 μ in diameter. Chips of wood are first macerated either by standing for several days in cold 5% chromic acid or by alternate treatments with chlorine water and hot 3% sodium sulphite. The tissue softened in this way is then shaken vigorously with glass beads until the cells are completely separated. After this, a suspension of the cells in distilled water is run over a series of microscope slides smeared with albumen fixative and allowed to dry down on the slide. Immersion of the slide in alcohol serves to harden the fixative. A sharp microtome knife is then slid over the surface of each slide, removing all loosely attached cells and all large conglomerations and cutting away the upper walls of many cells which are affixed firmly to the slide by their lower walls. The slides are then prepared for examination under the microscope in the normal way, no stain, of course, being required. With practice, it is possible in this way to achieve 100 or more single walls on each slide. Following the methods described earlier (Chapter IV) the angle θ between the m.e.p. and the cell length is then determined for a number of cells, normally 50, sufficiently large to give a representative mean.

The results are recorded as the mean plus or minus the standard error (*i.e.* the standard deviation divided by the square root of the number of observations).

A series of such determinations is presented in Table VI for tracheids and for a variety of fibres; these will be examined in detail later on. At the moment we are interested only in the general picture of tracheid structure. We note that the m.e.p. is always inclined to the length of the cell through a considerable angle which varies rather widely from sample to sample in a way we shall have cause to discuss later on. Now whenever a *whole* cell is examined the effective m.e.p. is always either longitudinal or transverse, never tilted in this way; in fact this difference between double and single walls is one way in tracheids by which cells which have been cut open may be detected. Referring back to Fig. 3 this must mean that the m.e.p. of the upper wall in any cell makes the same angle to cell length as that of the lower wall but in the opposite direction, *i.e.* the m.e.p. is arranged spirally round the cell.

Such a spiral arrangement of the m.e.p. can be interpreted provisionally in terms of the orientation of the cellulose chains. The cell wall may be built up of chains all lying parallel to the m.e.p. and this possibility receives strong support from other observations which can be made in these cells. Striations, for instance, can often be observed in the walls and, remembering the close connection found in *Valonia* and *Cladophora* between striation direction and chain direction, we could hazard a guess that here too the striation direction conforms to the direction of the chains in the layer in which the striations are visible. Now it is clear from Table VIA that the directions of the m.e.p. and the striations are never far removed from each other. Equally the same thing is true if we compare the run of the slit mouths of bordered pits and the m.e.p. Now if there were present layers of any considerable extent whose cellulose chains ran in some direction other than that of the striations, then this correspondence could not hold. This therefore leads immediately to the conclusion that the bulk at least of the walls of these cells is built up of chains running at the angle given by the m.e.p. It is, of course, not possible to be sure that other directions are absolutely missing. There are some discrepancies in Table VIA between striation direction and m.e.p. and, in any case, the errors involved in measuring either (of the order of $1/2°$) are too great to allow any certainty about the exact correspondence. But it is, at any rate, certain that the m.e.p. does give the position of the bulk of the chains in the wall within the limits of a rather small error. The chains of cellulose in conifer tracheids, therefore, and of other elongated cells in which the

TABLE VI

The angle θ between the cellulose chains and cell length as determined by a variety of methods

Cell type	*Method of Determination*		*θ*		*Authority*
			Range	*Average*	
Tracheids	m.e.p.	A.R.*	*Cedrus* sp.		
		2	36·0–67·0	56·0±1·0	
		4	36·6–70·0	49·3±1·1	
		6	37·0–62·2	50·4±1·0	
			Larix leptolepis		
		2	36·0–73·0	53·3±1·2	
		4	35·0–69·4	50·8±1·2	Preston (1934)
		6	34·0–58·4	45·5±0·7	
			Abies sp.		
		2	42·8–68·8	53·3±1·0	
		4	34·8–64·8	47·5±1·0	
		6	35·8–62·4	49·8±0·9	
		8	30·0–52·8	44·7±0·9	
		11	23·0–47·6	34·7±0·8	
			Pinus longifolia		
		2	39·0–74·0	56·3±1·3	
		4	34·5–61·0	43·7±1·1	Misra (1939)
		6	22·0–57·0	35·5±1·2	
	X-rays		*Pinus sylvestris*		
		2	—	36·2	
		4	—	30·1	
		8	—	19·3	Preston (1948)
		11	—	14·0	
Fibres			*Cannabis sativa* (hemp)		
	m.e.p., striations		0·0–5·0	2·3±0·3	Kundu and
			0·0–6·0	2·0±0·3	Preston (1940)
			Corchorus capsularis (jute)		
	m.e.p.		0·0–23·0	7·9	Preston (1941)
			Sisal		
	m.e.p., X-rays		8·0–32·3	20·4±7·2	Preston and Middlebrook (1949)
				18°	
			Bamboo		
			Dendrocalamus longispathus		
	m.e.p.		2·0–8·0	5·0±1·2	
			Dendrocalamus strictus		Preston and
			3·0–7·0	6·4±1·7	Singh (1950)
			Bambusa arundinacea		
			2·0–8·0	5·4±1·3	
			Melocanna bambusoide		
			7·0–14·0	10·4±1·9	

*A.R.=annual ring.

TABLE VI—*contd.*

Cell type	*Method of Determination*		*θ*		*Authority*
			Range	*Average*	
Collenchyma cells	m.e.p.		*Petasites vulgaris* 0·2–4·2	2·0±3	Preston and Duckworth (1946)
			Heracleum sphondylium 0·0–1·5	0·6±0·08	Majumdar and Preston (1941)
Vessels	m.e.p., striations and slit pits		*Quercus alba* 90–47·0	—	Preston (1939)
			Fraxinus americana 71·0–51·0	—	
			Quercus borealis 88–82	—	

TABLE VIA

Correspondence between direction of m.e.p. and of striations in elongated cells. Both directions are defined by the angle they make to cell length

Cell type	*m.e.p.*	*Striation direction*
Fibres		
Hemp		
primary	3·0±0·4*	2·1±0·3
secondary	2·3±0·3	2·0±0·3
Tracheids		
Pinus insignis	23·5±1·8†	25·0
(Individual	15·0±0·5	16·5
determinations)	19·0±2·0	16·5
	16·6±1·0	16·5
	15·5±1·0	16·5
	4·7±2·0	2·5
	20·0±2·8	17·8
	26·0±2·0	26·0
	18·9±1·0	20·8
	42·9±1·0	46·8
	28·5±2·0	28·5

* Standard error.

† The following m.e.p.s were calculated from the optical data on *whole walls* to exclude the effects of the primary wall. The error is the sum of the experimental errors, not the standard error.

m.e.p. is tilted in this way, must form a spiral round the cell. The spiral is not, however, uniform from cell to cell, and within any small sample of wood or any other fibrous tissue there occurs a wide range of spiral angles. With this information regarding the structure of these cells we can now proceed to examine the X-ray diagram; we shall return later on to the optical properties when we wish to examine the wall in further detail.

The X-ray diagram of conifer wood. The spiral diagram

When a chip of wood some 1 mm. thick is arranged in an X-ray spectrometer so that the X-ray beam traverses the specimen in the radial direction, then a diagram is obtained like those illustrated in Plate VII, Fig. 1. These are clearly very similar to those of ramie and of hemp (Plate II, Fig. 3) where we know that the cellulose chains run longitudinally. It is therefore immediately clear that here too the chains often run almost longitudinally and that the closer resemblance of later annual rings (Plate VII, Fig. 1(*c*)) to ramie and hemp means that in these later rings the chains are much more nearly parallel to cell length. This is found invariably to be true (see p. 159). The arcs are, however, commonly much more diffuse than in ramie and in particular they have, with the exception of the outermost wood of older stems, a much greater lateral spread; in the innermost annual rings, in fact, they are spread almost into a complete circle (Plate VII, Fig. 1(*a*)).

At first sight one might conclude that the cellulose chains in wood, while tending to lie longitudinally as in ramie and hemp fibres, have a much greater angular dispersion about this direction, and there is often nothing in the diagram to indicate that this interpretation is in error. Now, however, that we have the fact recorded above that the m.e.p. of single walls is usually tilted through a very considerable angle to cell length, we can see that this interpretation cannot, in general, be correct. In fact, as we shall see, the diagram of a spirally wound cell resembles rather closely that of cells whose chains have a wide dispersion about the longitudinal direction. In interpreting the diagrams both angular dispersion and spiral organization have to be taken into account and we can perhaps best appreciate the factors involved in the following way. The argument here becomes rather mathematical. It is supported, however, by X-ray diagrams of model spirals (Plate VI) which can be consulted *in lieu* of the mathematics.

The evolution of the fibre diagram can be followed with the aid of spherical projection and the pole figure (Fig. 41). A crystal is imagined as lying at the centre of a sphere, and the point at which the normal

to any set of molecular planes intersects the spherical surface is called the *pole*, *P*, of these planes. The conditions of reflexion of X-rays from the set of planes are defined by the reflexion circle, *PQRS*, the locus of the pole when the planes are inclined at the glancing angle θ to the X-ray beam. In Fig. 41 the pole *P* is drawn in a position for reflexion. The reflecting positions are thus given by the points at which the reflexion circle intersects the locus of the pole. If the crystal is rotated about *Mm* the locus of the pole is two small circles L_1l_1 and L_2l_2, which therefore intersect the reflexion circles at four points, *P*, *Q*, *R* and *S*

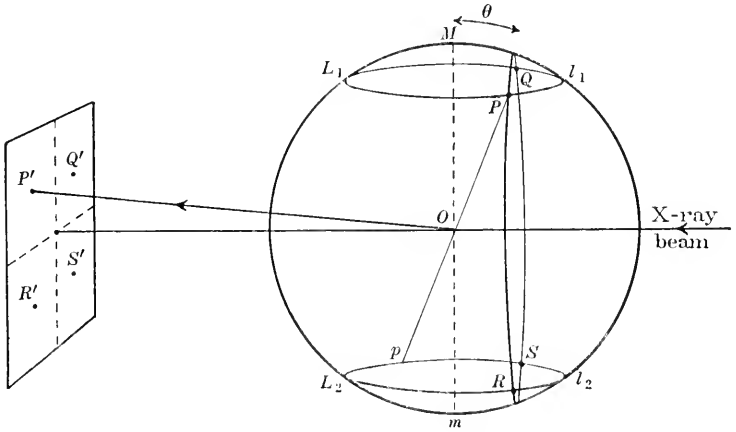

FIG. 41. For explanation, see text.

corresponding to four symmetrically disposed spots *P'*, *Q'*, *R'* and *S'* on the photographic plate. It will simplify matters considerably if only those planes of most importance are here considered, *i.e.* planes parallel to the direction of rotation. There are then only two positions for reflexion, lying along the equator of the plate and equidistant from the centre (Fig. 42(*a*)).

If now the crystal is replaced by a bundle of parallel fibres, in which the cellulose chains run longitudinally, arranged to lie parallel to the rotation axis *Mm*, then Fig. 42(*a*) gives also the derivation of diffraction spots from planes lying parallel to the cellulose chains. The major difference between this ideal geometrical pattern and that of a real natural fibre is merely that no part of the fibre represents a real perfect crystal. Its cellulose component consists of innumerable minute regions —the micelles—in which the chains are strictly parallel and arranged in a regular manner, separated by "intermicellar" spaces in which the chains run from micelle to micelle in a more random fashion. These

micelles are elongated and on an average lie parallel to the length of the fibre; they are, however, tilted to a greater or less degree to this common line of orientation, *i.e.* they have *angular dispersion*, and this can be allowed for by allowing the axis *Mm* to wobble slightly as it rotates. The intersection of the axis with the projection sphere then describes two polar caps limited by two small circles *MM'* and *mm'*, and the locus of the pole is a family of circles of which two are represented in

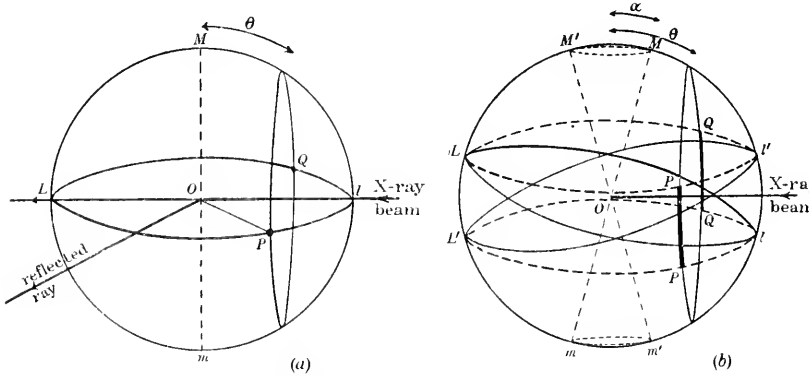

FIG. 42. For explanation, see text.

Fig. 42(*b*); the spots are therefore drawn out into two arcs *PP*, *QQ*, which are more intense at their centres.

Now to derive from this construction the pattern to be expected from a set of planes parallel to the cellulose chain direction in a fibre wound with a spiral, one has merely to remember that the spiral is formed in effect by a tilt of the micelles through a constant angle to the longitudinal, in the plane of the fibre wall. Taking any small element of the wall, therefore, the axis of rotation is tilted through some angle *S*, and the effect may be seen in Fig. 42(*b*) if α is replaced by *S* in the diagram. The circle *Ll*, the locus of the pole to which *Mm* is normal, is tilted through the same angle *S*, and its intersection with the reflexion circle is therefore depressed. To represent the whole figure the axis *Mm* is rotated about the direction of the fibre length, the axis traces out the hollow cone *MmOM'm'*, and the locus of the pole *P* becomes the belt *LlL'l'* bounded by two broken small circles in Fig. 42(*b*). The lateral spots are again therefore drawn out into two arcs whose lengths depend on the value of the angle *S* of the spiral. Clearly, however, the intensity distribution along the arcs will be different from that referred to in the arcs derived above for angular dispersion. The cone

MmOM'm' is now hollow instead of solid, and it is no longer evident that the centre point of each arc will have the highest intensity. On the contrary, it can be shown that the intensity is now highest at the *ends* of the arcs; this may be seen quite simply in the following way. In Fig. 43(*a*) let S be the spiral angle (cp. Fig. 43(*b*)), ϕ the angular distance of the pole at P from the spiral axis Aa, δ the angle between the great circles MPm and APa and PN the perpendicular from P to the spiral axis.

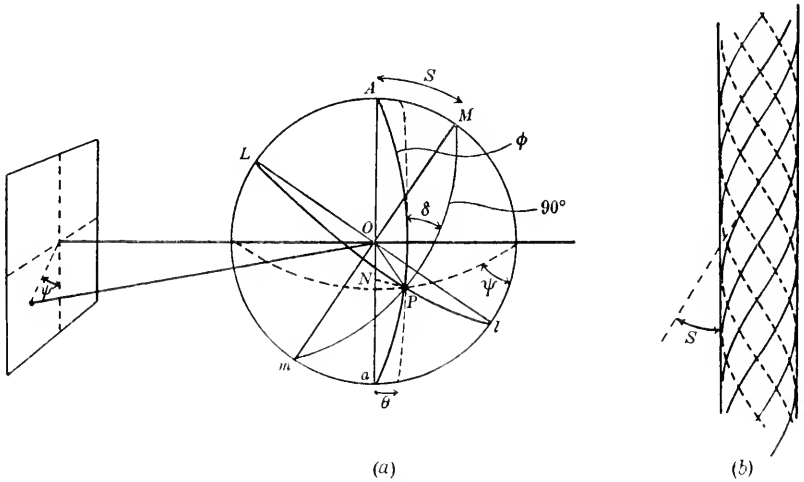

FIG. 43. For explanation, see text.

Then since the great circle Ll is a line of equal pole density, the density at P on the surface of the sphere is inversely proportional to PN and $\sin\delta$, *i.e.*

$$D_p \propto 1/(\sin\phi \sin\delta),$$

but
$$\sin\delta = \sqrt{(1-\cos^2 S/\sin^2\phi)},$$

therefore

$$D_p \propto 1/\sqrt{(\sin^2\phi - \cos^2 S)}.$$

The corresponding intensity in the photograph I_p, is proportional to Dp, but ϕ must be converted into a vector measurable on the photograph. This can be done by substituting

$$\cos\phi = \cos\theta \cos\psi.$$

Hence

$$I_p \propto 1/\sqrt{(1-\cos^2\theta \cos^2\psi - \cos^2 S)}.$$

I_p is therefore a maximum at the points

$$\psi = \cos^{-1}(\sin S/\cos\theta), \quad 180 - \cos^{-1}(\sin S/\cos\theta), \quad ..(1)$$

since at these points the intensity is theoretically infinite. This means that the intensity is the greatest at the ends of the arcs. Each arc therefore breaks up into two spots and the diagram consists of four spots, symmetrically placed, instead of two lateral arcs. If to this be added the angular dispersion of the micelles it is seen that the spiral photograph is liable to be somewhat complex. Depending on the value of the S, the four arcs may overlap on the equator, giving a false impression of a real equatorial spot, or even, if the dispersion is great enough, fuse into two meridional arcs. Finally, overlap may occur in both positions, giving eight apparent arcs in all, only four of which are real. Several further points appear from the equation for intensity:

(1) Provided the dispersion is not unduly high, a fibre of circular cross-section gives a photograph in which the expected four arcs appear symmetrically placed.

(2) As S, the spiral angle, increases, the four spots approach the meridian more and more closely. As this happens, the danger of an overlapping of these arcs along the meridian, giving a spurious arc, becomes greater. At a value of S given by $\cos S = \sin\theta$, the spots fuse into two meridional arcs (because at this value of S, $I_p = \infty$ only when $\psi = 0$ or 180°). Taking the planes of 3·9 Å., 5·4 Å. and 6·1 Å. spacing, the critical values for S appear to be as follows:

	S (limiting)	
Spacing	No dispersion	Dispersion ±5°
3·9	78·5°	73·5°
5·4	81·8°	76·5°
6·1	82·8°	77·5°

Hence all fibres whose spirals are flatter than $S = 73{\cdot}5$, say, for the 3·9 arc, or than the corresponding figure for the other two arcs, will give two meridional spots instead of four lateral arcs. In a mixture of fibres in which S varies sufficiently widely, therefore, one would expect spirals flatter than those given in the above table to contribute to the meridional arc, and the 3·9 arc should therefore appear intense out of proportion to the 5·4 and 6·1 arcs. It is interesting to note that this is the case here, and that in tracheids generally spirals do occur with sufficiently great values of S.

(3) If the spiral is flat, *i.e.* $S = 90°$, then the intensity equation reduces to

$$I_p \propto 1/\sqrt{(1-\cos^2\theta\cos^2\psi)}.$$

At $\psi = 0$, therefore, the intensity is proportional to $1/\sin\theta$ and at $\psi = 90°$

it is proportional to unity. One has, then, the arcs drawn out into a continuous circle with the intensity rather greater along the meridian. Looking at it another way, a set of chains running round a tracheid in transverse circles or a very flat spiral, would give two very wide arcs in the meridional position whose arms would meet at the equator to give a complete ring.

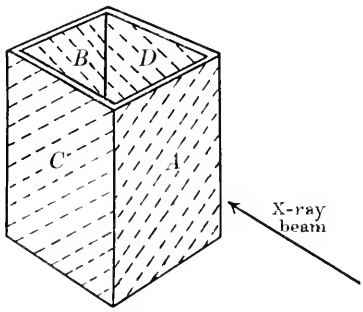

FIG. 44. For explanation, see text.

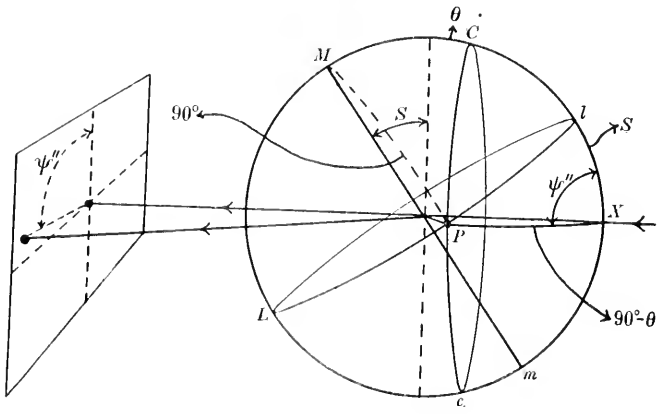

FIG. 45. For explanation, see text.

Thus in the diagram given by a bundle of fibrous cells with spiral cellulose chains four major arcs could be expected from the planes of spacing 3·9 Å., and four minor arcs, two along the equator and two along the meridian. For the sake of completeness it may also be noted that equatorial arcs may be derived in another way. Suppose the tracheids are rectangular in cross-section, with two opposite faces normal to the beam (Fig. 44). Then the walls *A* and *B* will clearly give four major arcs in diagonal positions and perhaps spurious equatorial or meridional arcs or both. The walls *C* and *D*, however,

will give arcs which are nearly equatorial. Their actual positions may be determined in the following way. Figure 45 shows the projection circle and pole figure appropriate to the case, the symbols having the meanings already defined. The relation $\cos \psi^{\parallel} = \tan S \tan \theta$ is obtained from the spherical triangle *MPX*. For the arcs given by the walls *A* and *B* normal to the beam the angular distance of each from the meridian is $\psi^{\perp} = S$. One has to compare $\psi^{\parallel}$ with $\psi^{\perp}$. This can be done with specific examples and some of these are shown graphically in Fig. 46, where the larger solid circles correspond to $\psi^{\perp}$ and the smaller ones to $\psi^{\parallel}$. Even with a spiral as steep as $S = 30°$ the arcs corresponding to the lateral walls are displaced by 7° from the equator and therefore separated by 14°. Sufficient dispersion would cause these arcs to overlap along the equator, but these fused arcs would then of necessity be very diffuse, ranging over an angular distance of the order of 35°. Except, therefore, for the steeper spirals one should not expect a sharp equatorial arc from the lateral walls; and in these steeper spirals an arc of this type would probably be masked by the arcs from walls *A* and *B* which then approach the equator.

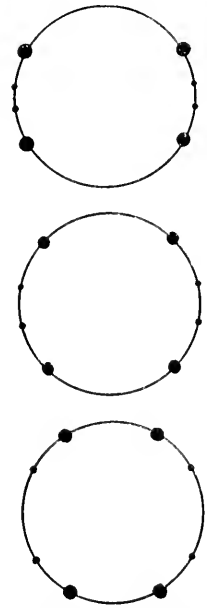

FIG. 46. For explanation, see text.

Before proceeding to examine the diagrams of wood in the light of this discussion of the fibre diagram, it will be as well to compare the predictions with the actual diagrams of spirals of known spiral angles. A series of such diagrams are presented in Plate VI. These were prepared for me by Dr. V. Ranganathan of the Forest Research Institute, Dehra Dun, India, while working in my laboratory, in the following way. Filaments of artificial silk with remarkably perfect orientation (see Plate VI, Fig. 1) were carefully wound around a horse-hair stretched between the manuals of a micro-manipulator in such a way that the angle between the filament and the hair remained constant over a distance of several millimetres. In this way a series of spirals with known spiral angles of approximately 0° (parallel bundle), 10°, 20°, 30°, etc., up to 90° (flat spiral) were prepared, each of them less than 0·5 mm. in diameter and therefore completely covered by the beam issuing from the conventional 0·5 mm. slit in an X-ray spectrometer. The diagrams of these spirals were obtained in turn, with the beam as usual normal to the lengths of the spirals. It will be seen that, in the

first place, there is good qualitative agreement between the diagrams obtained and the predictions from theory, in that the lateral arcs do split up into 4 spots symmetrically disposed about the centre of the diagram and that in the flatter spirals these fuse into two meridional arcs. Table VII, giving the values of the angle ψ as actually observed on

TABLE VII

The angular distance, ψ, between the arcs on the X-ray diagrams of model spirals

Angle of spiral	*Value of ϕ in degrees*			
	Spacing 4·0 Å.		*Spacing* 7·3 Å.	
(obs.) (S)	*Observed*	*Calculated from S*	*Observed*	*Calculated from S*
9·5	77·1	80·3	78·0	80·5
19·8	67·0	69·8	68·5	70·1
29·3	58·0	60·3	59·6	60·5
40·7	44·6(?)	48·5	47·7	49·0
41·8	45·2(?)	47·2	46·2	47·9
56·5	30·0	31·6	32·1	33·0
68·2	19·2	18·9	19·7	21·0
76·3	8·7	8·0	11·4	12·3

the diagrams and as calculated from equation (1), p. 123, makes it further clear that the agreement is good also in a quantitative sense. There can therefore be no doubt as to the general validity of the considerations put forward above. The fusions of arcs referred to do not make themselves evident in these model spirals because the sample of artificial silk chosen to give the best check against the theory had naturally as little angular dispersion as possible; but parallel diagrams of an inferior material did in fact give such fusions in which, in particular, the four spots became fused into two equatorial arcs although these cannot be recorded here for limitations of space.

Such fusions are, however, very evident in the less perfectly crystalline material found in wood. Here, the lateral 3·9, 5·4 and 6·1 arcs are sometimes separated into four spots (Plate VI, Fig. 2), although often these are caused to fuse, as a result of high angular dispersion, into two equatorial arcs. One feature of these diagrams is always, however, most striking. Whenever these lateral arcs are wide, *i.e.* when the spiral is relatively flat, then two meridional arcs also appear at the same distance from the centre of the diagram. These are very evident in the diagram presented here in Plate VI, Fig. 3. Attention was first called

to these by Bailey and Berkeley (47(*d*)) who regarded them as evidence for the presence in wood of *transverse* cellulose chains which would, of course, produce arcs in exactly this position. We can see now, however, that it is possible that these arcs represent nothing more than the fused ends of the widely spread equatorial arcs. This has been shown, in fact, to be the case (47(*e*)). We can verify that such arcs are spurious here in a most convincing way by comparing Plate VI, Fig. 3 with Plate VII Fig. 1. In the former the lateral arcs are widely spread and the meridional arcs are very evident. In the latter the lateral arcs are much more sharp and now there is no trace of any meridional arc. The layering of the cells as seen under a polarizing microscope in transverse section, which Bailey associates with the presence of transverse cellulose chains (p. 114) and therefore with the meridional arcs, is equally evident in both samples of wood. It is therefore certain that these arcs have no connection with the structural features underlying the optical heterogeneity, and re-examination of the three diagrams obtained from different regions of one piece of wood (Plate VII, Fig. 1) makes it quite clear that the meridional arcs are indeed spurious. For, although the make-up of the cells in the three samples of wood must be very much the same, a meridional arc is noticeable in the diagram of wood from the third and fourth annual ring but not from the tenth. Here again it is clear that the presence of a meridional arc is associated with a spreading of the lateral arcs. In all these cases the apparent intensity of the spurious 3·9 Å. is intensified by the fusion of the 021 arcs along the meridian and the meridional arc itself is in reality rather weak.

There is thus no satisfactory evidence for the presence of flat spirals in conifer tracheids, and this lends support to an earlier statement (47(*c*)) that in X-ray diagrams of single tracheids there is no sign of transverse orientation. There can therefore be no doubt but that the X-ray diagram corresponds to only one spiral set of cellulose chains, and we shall see in a moment that this is a most puzzling feature of structure here. For consideration of the detailed optical properties of the tracheids wall has now made it quite clear that, although chains oriented in the transverse, or even approaching the transverse, plane as postulated first by Bailey are absent, the outer and inner layer in the wall do differ markedly in chain direction from that in the central layer.

Crossed fibrillar structure in tracheids and fibres

Although the X-ray evidence and to a large extent also the optical evidence thus seemed fairly clear, there were still some unsatisfactory features of the optical properties of tracheids which had been noted

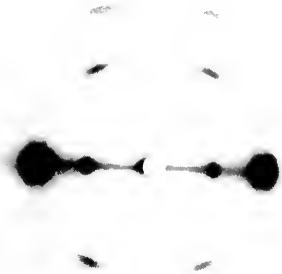

FIG. 1. Parallel bundle (0° spiral).

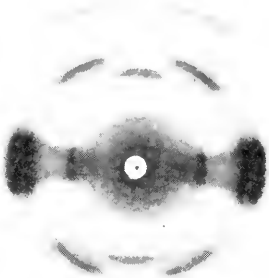

FIG. 2. 9·5°.

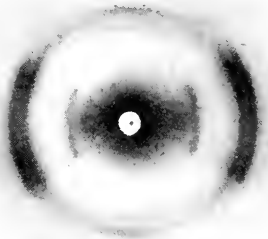

FIG. 3. 19·8°.

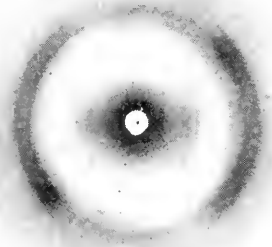

FIG. 4. 40·7°.

X-ray diagrams of spirals constructed using filaments of well-oriented artificial silk. Each spiral was less than 0·5 mm. diameter and therefore lay within the X-ray beam. The angles given are those between the filament and the spiral axis. Spiral axis parallel to long edge of page.

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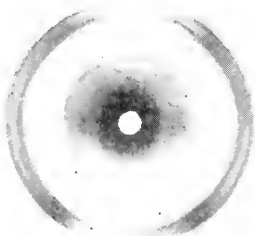


FIG. 5. 56·5°.

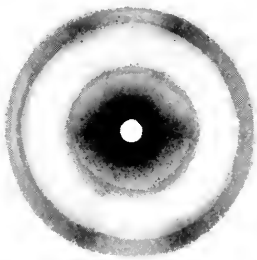


FIG. 6. 68·2°.

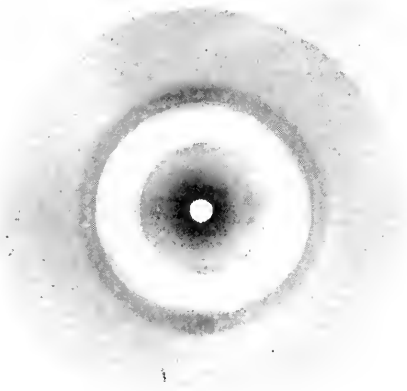
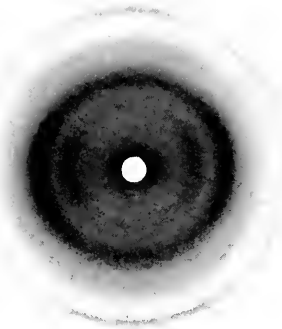
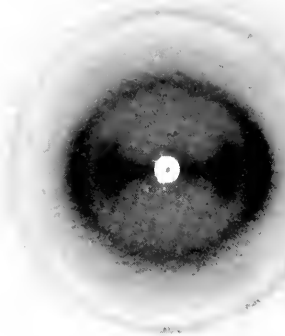


FIG. 7. 90° (flat spiral).

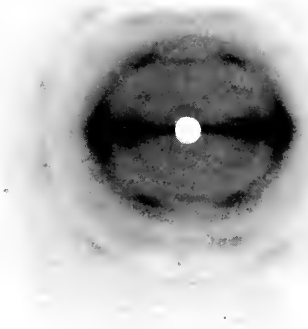
X-ray diagrams of spirals constructed using filaments of well-oriented artificial silk. Each spiral was less than 0·5 mm. diameter and therefore lay within the X-ray beam. The angles given are those between the filament and the spiral axis. Spiral axis parallel to long edge of page.



(*a*) 3rd annual ring.



(*b*) 4th annual ring.



(*c*) 10th annual ring.

FIG. 1. X-ray diagrams of late wood from one transverse slice of a trunk of *Pseudotsuga taxifolia*. Beam perpendicular to grain, CuK_{α}. Note that as the lateral arcs become shorter tangentially, the intensity along the meridian diminishes.

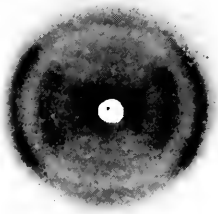


FIG. 2. X-ray diagram of wood of *Juniperus virginiana*, beam perpendicular to grain. Note the lateral arcs are more intense towards their ends, as predicted by the theory of the spiral diagram. Note also the overlap along the meridian to give spurious meridional arcs.

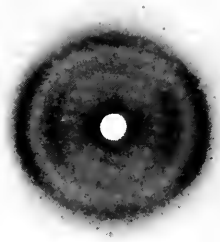


FIG. 3. X-ray diagram of a sample of *Pinus sylvestris*, late wood, 4th annual ring. The lateral arcs are barely intensified at their ends, indicating a wide dispersion of spiral angle in the specimen. The spurious meridional arc is prominent.

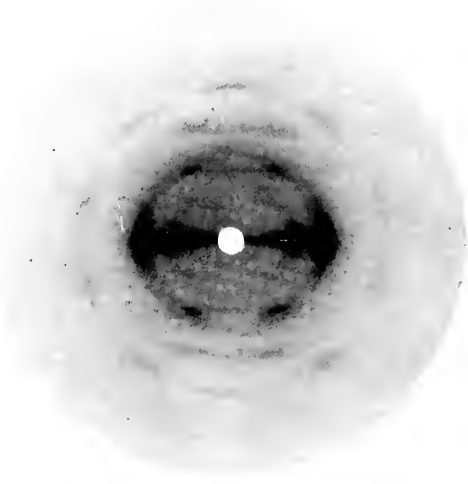


FIG. 4. X-ray diagram of a sample of *Abies* sp., 10th annual ring, late wood. Note the lateral arcs are rather short and there is no overlap along the meridian.

The photographs, figs. 2, 3 and 4 similarly demonstrate, therefore, the association between the appearance of a meridional arc and the tangential spread of the lateral arcs.

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by A. B. Wardrop of the Forest Products Division, C.S.I.R.O., Australia, in comparison with, for instance, those of vessels. We shall not discuss these here; they will be found set out fully in the papers which will be quoted later, but they did eventually stimulate the writer in collaboration with Wardrop to a further optical analysis of tracheid and wood fibres (48(*a*)), and at the same time to attempt to generalize the investigation by looking also into the structure of sisal fibres (48(*b*)) and bamboo (48(*c*)). Since all these elongated cells were found to tell more or less the same story throughout, they may perhaps be dealt with here together. In all major points which we shall discuss in the rest of this chapter any one of these fibres (and probably any fibrous cell) may be chosen as example.

Although it is the intention to follow through the history of conifer tracheids since we do know now much more about them than about any other single cell, we may begin with the simplest case analytically, the case of sisal fibres.

Sisal is simpler from the present point of view for this reason. Normally, with fibres, we have to accept the mature fibre as the only easily available material, and then to try to deduce the structure of the various layers from the properties of the whole wall. With sisal, however, it is easily possible to obtain large quantities of fibres in which the outer layer only is present and thus to examine this layer separately. The sisal plant is monocotyledonous and the fibres occur in the leaves. In common with other monocotyledons the leaves continue to grow at the base throughout the life of the plant, so that in any one leaf we can find all stages in fibre development from expanding fibres, at the base of the leaf, with only the primary wall, through fully elongated fibres with the outer layer only of the secondary wall, to fully mature fibres farther towards the tip. It is therefore merely a matter of taking material from the correct level above the base of the leaf to find fibres in any desired condition. The results of examination of such material are quite striking. The X-ray diagram of fibres carrying only the outer layer of the secondary wall (Plate VIII, Fig. 1) is quite different from that of fully mature fibres (Plate VIII, Fig. 2), and looking back at the diagrams of model spirals we can see immediately that the spiral on the immature fibres is much flatter than that on the mature fibres. The spiral angles which may be derived from the diagrams are given in Table VIII, together with a number of other features of these cells. The following points should especially be noted. Firstly, the immature and the mature fibres are of almost exactly the same (average) length; there can be no question here of the length effect we shall find later in other

TABLE VIII

Fibre length and chain orientation in sisal fibres

Leaf	*Length of leaf*	*Distance from base at which observations are made*	*FIBRE LENGTH*			*MAJOR EXTINCTION POSITION*			*Angle of pits*	*Angle from X-ray data*	*Percentage cellulose*
			Number of observations	*Range (mm.)*	*Mean (mm.)*	*Number of observations*	*Range*	*Mean*			
B.3	153 cm.	0–3 cm.*	359	0·33–2·68	1·06±0·34					49°	65·4
B.3		54–58 cm.	158	1·15–5·15	2·58±0·78					18°	78·3
C.105	79·9	0–3 cm.*	165	1·23–5·16	2·78±0·82	31	26·4°–64°	40·2°±8·4°	39·5°	40°	77·3
C.105		10–13 cm.	181	1·27–4·96	2·78±0·89	31	8·0°–32·3°	20·4°±7·2°		18°	83·2
C.105		76·7–9·9 cm.	267	8·07–4·92	2·50±0·94					20°	83·2

* Outer layer present only.

elongated cells (p. 152) introducing any complications. Secondly, the birefringence (n_γ'—n_α) of the outer layer in transverse section is the same in immature and in mature fibres; this makes it rather certain that the outer layers in immature and mature fibres are essentially similar in general physical make-up (Table VIIIA). We can safely conclude, therefore, that the diagram of immature fibres represents the condition in the outer layer both of immature and of mature fibres, and that the steeper spiral deducible from the diagram of the mature fibres refers to the thick central layer only. There can thus be not the slightest doubt here but that the outer layer is composed of cellulose chains lying in a fairly flat spiral, the angle to cell length being of the order of 40°, while the central layer later deposited upon it is composed of chains lying much more steeply, with an average inclination to cell length of about 20°.

It is therefore again very curious that there is, in the diagram of the mature fibres, no record of the orientation which we know must be present in the outer layers; the "mature" diagram is not merely a superposition of the diagrams of the outer and the central layers but corresponds to the central layer only. This is exactly the condition we have met in conifer tracheids. There for the time being we may leave the sisal fibres, though we shall have cause to return to them again.

Turning next back to the tracheids, it has been found possible to establish more or less the same type of structure even though mature cells only are available; and in fact to add something to the sisal story which may help to explain the curious anomaly just mentioned. The method used takes advantage of the fact that conifer tracheids are approximately rectangular in transverse section, with two opposite walls lying tangentially in the stem and two radially. In any small section (containing nevertheless hundreds of cells) the tangential walls are almost exactly coplanar—sufficiently nearly so, at any rate, for present purposes. Consider one such cell (Fig. 47). In transverse section the chains (marked by dotted lines) will be seen in the same "perspective" in all four walls and these are equally birefringent. When, however, the cell is cut in some other plane $XXYY$ then one wall AB

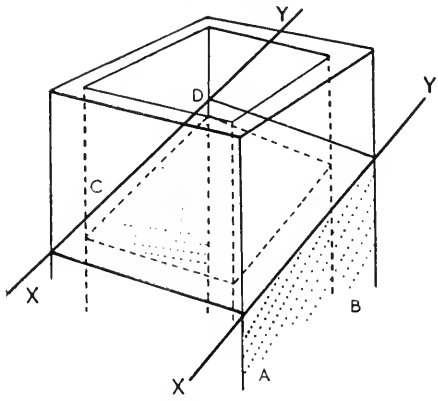


FIG. 47. For explanation, see text.

TABLE VIIIA

Wall thickness and birefringence in sisal fibres
(*Leaf* C106 *is closely similar to leaf* C105 *in Table VIII*)

Leaf	*Length of leaf*	*Distance from base*	*Number of observations*	*WALL THICKNESS in μ*			*BIREFRINGENCE OUTER LAYER*			*BIREFRINGENCE INNER LAYER*		
				Total	*Outer layer*	*Inner layer (by difference)*	*Number of observations*	*Range*	*Mean*	*Number of observations*	*Range*	*Mean*
C.106	74·2 cm	1 cm.	30	0·68–0·15	0·68–0·15	0·00	30	0·0102–0·0237	0·016			
C.106	74·2cm.	12 cm.	33	1·26–0·11	0·66–0·18	0·60	22	0·0087–0·0191	0·015	22	0·0044–0·0078	0·0064

will become much more birefringent than the opposite wall *CD*, since in the former wall the plane of section is more nearly parallel to the direction of the cellulose chains than it is in the latter. When the plane

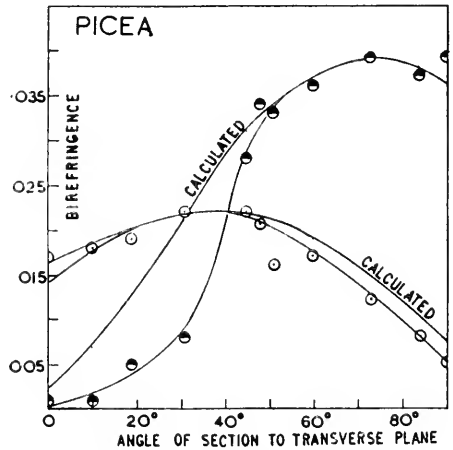


FIG. 48. For explanation, see text.

of section is tilted to the transverse through an angle $(90°-\theta)$, the birefringence in *AB* will clearly be at a maximum. Hence it is possible to measure the angle θ by plotting the birefringence against the angle

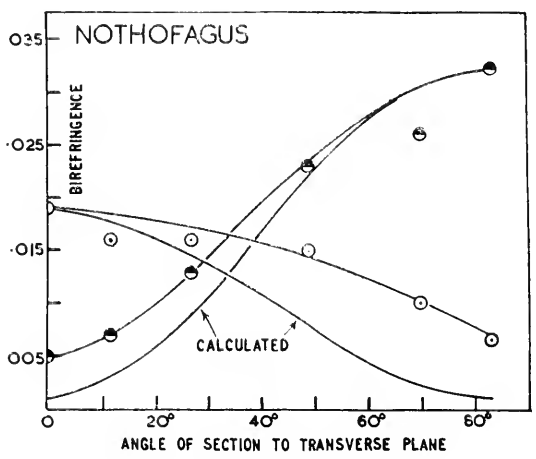


FIG. 49. For explanation, see text.

of sectioning for a series of sections at different angles to the transverse, and reading off the angle for maximum birefringence. If, now, the different layers in a wall have different spiral angles, then the maxima

will occur at different angles of tilt, and the spiral angles can be measured. This is the principle upon which the observations in conifer tracheids were made.

A series of sections were cut at various angles to the longitudinal radial plane so that the walls examined were the tangential walls. In each section a sufficient number of measurements of birefringence were made to give a reasonably accurate average. This was done by measur-

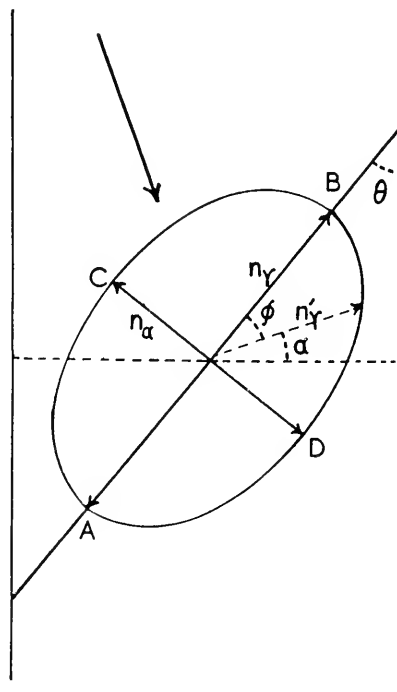


FIG. 50. For explanation, see text.

ing the phase difference in a de Sénarmont compensator as already described (p. 69) using sodium light, and subsequently measuring the thickness of the section (always of the order of 5 μ) by turning over a few cells on their edges. The results for both outer and central layers are given graphically in Fig. 48 for tracheids of *Picea* sp. and in Fig. 49 for the fibres of *Nothofagus cunninghami* (48(*a*)). In each case a curve is also included which gives at any angle of sectioning the birefringence which could have been expected, calculated from the observed maximum birefringence. This curve can be calculated in the following way. Let *ABCD*, Fig. 50, be the trace on the wall surface of the index ellipsoid

corresponding to a maximum birefringence of $n_\gamma - n_\alpha$, and suppose the section to be cut at any angle ϕ to the major axis of the ellipse and therefore to the preferred orientation of the cellulose chains. Then when the wall is examined in the direction marked by the arrow, at right angles to the surface of the section, the major refractive index is n_γ'. From the equation of the ellipse, we can say:

$$\frac{(n_\gamma')^2 \cos^2 \phi}{n_\gamma^2} + \frac{(n_\gamma')^2 \sin^2 \phi}{n_\alpha^2} = 1,$$

or

$$(n_\gamma')^2 = \frac{n_\gamma^2 n_\alpha^2}{n_\gamma^2 + (n_\gamma^2 - n_\alpha^2) \sin^2 \phi}.$$

Hence, since the value of the minor refractive index is invariate (the wall being uniaxial), the birefringence at this angle of sectioning is

$$n_\gamma' - n_\alpha = \frac{n_\gamma n_\alpha}{\sqrt{n_\alpha^2 + (n_\gamma^2 - n_\alpha^2) \sin^2 \phi}} - n_\alpha.$$

The birefringence at any angle $\phi = 90° - (\theta + \alpha)$ where α is the angle between the plane of sectioning and the transverse plane, can therefore be calculated.

The first point to note is that the angles of maximum birefringence are not the same in outer and central layers for either type of cell. In *Picea*, the micelles in the outer layer are, on an average, oriented at an angle of about 50° to the length of the cells while in the central layer the angle is 18° (comparing favourably with the value of 20° calculated from the X-ray diagram). In *Nothofagus* it is somewhat difficult to determine the precise angle in the outer layer—it must lie between about 90° and 60° to the length of the fibre—but it is certainly much greater than the 10° (the value also determined from the X-ray diagram) of the central layer. It seems therefore to be established, by the only type of observation which could firmly establish such a result, namely by observation of the physical properties of undistorted walls, that the micelles in the outer layers of tracheids and wood fibres while not transverse, do at least form a spiral much slower than that in the central layer.

On the other hand it is equally clear that such a change in the net orientation from layer to layer is not the only factor involved in determining the optical properties of these walls. Thus, in the central layers, the birefringence of the cellulose is of the order of 0·04 as compared with the value 0·06 to 0·07 recorded for cellulose in ramie, cotton, etc. This lower value can be largely attributed to the high lignin content of the walls. Thus, chemical analysis of wood samples shows that the cellulose content is about 60% on a dry-weight basis. From this figure,

using methods already described (p. 56) it can be shown that, if the birefringence of the cellulose micelles is actually 0·06 then the maximum birefringence of the lignified wall should be rather more than 0·04. From such figures alone it seems possible that the low birefringence here could be accounted for completely in this way. Other considerations, however, show that other factors must also be involved. For reference to Fig. 50 will make it clear that in transverse section the major extinction position of the walls should always lie parallel to the wall surface, and this is a point which has already been discussed (p. 65). In tracheid walls, however, it was observed by the writer many years ago, and confirmed during this particular investigation, that the m.e.p. often lies well away from this parallel position and, in fact, is sometimes normal to the wall surface. This could only arise through a very considerable angular dispersion of the micelles, and this must add to the depression of the value of the maximum birefringence.

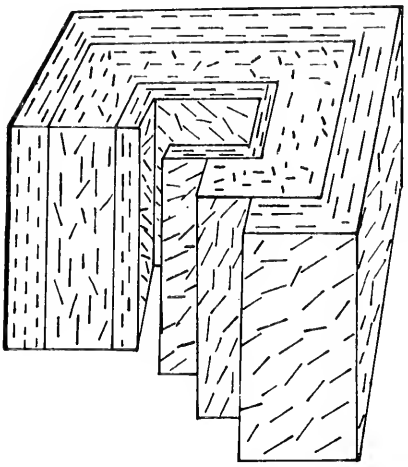


FIG. 51. Diagrammatic representation of the structure of the secondary walls of conifer tracheids. The broken lines represent the run of the cellulose chains.

In the other layer the same condition appears in a much more exaggerated form, since the birefringence is reduced to 0·02. At the moment, and until we know a good deal more about the chemical composition of this outer layer, it is not certain how far the observed difference in birefringence (0·04 as against 0·02) is to be accounted for by a difference in cellulose content. The low value in the outer layer, however, coupled with the observed discrepancies between the run of the observed and calculated curves in Figs. 48 and 49, makes it fairly certain that the cellulose here has an unusually high angular dispersion.

In these cells, therefore, the inner layer is dark not only because the micellar spiral is a steep one but also on account of the angular dispersion which reduces the birefringence to a level much lower than it would otherwise have been. Similarly the outer layer is much less bright in transverse section than one would expect, again on account of high angular dispersion and high lignin content. Our present

conception of structure in these cells is therefore to be represented as in Fig. 51. This structure has now been fully confirmed in the electron microscope (41 (*d*)).

Passing finally to the third cell type whose structure has now been worked out in sufficient detail, corresponding investigations were carried out in the writer's laboratory by Dr. K. Singh, of the Forest Research Institute at Dehra Dun, India, on samples of bamboo kindly

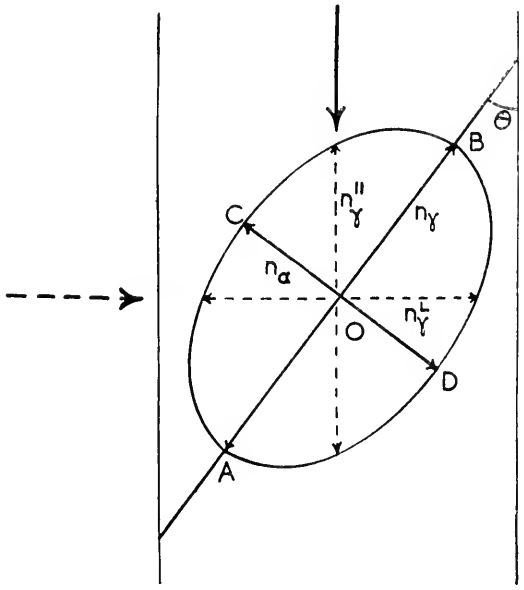


FIG. 52. For explanation, see text.

provided by the Institute. The bulk of the work was performed on the species *Dendrocalamus strictus* but the observations have mostly been corroborated with other species also (*D. longispathus*, *Bambusa polymorpha*, *B. arundinacea*, *Melocanna bambusoide*, and *Neuhouzea Dulloa*). These fibres, like sisal, are monocotyledonous but, unlike any of the cells studied up till now, they develop from the same ground meristem as parenchyma cells, so that they form an interesting contrast. The problem involved is, however, a much more difficult one. The fibres cannot be obtained in the immature condition, as could the fibres of sisal, and they are arranged in much too haphazard a fashion to allow the use of the method found so satisfactory for tracheids and wood fibres. Recourse has thus to be made to a thorough optical analysis.

Suppose, for simplicity, that we could determine accurately the birefringence of any layer in the wall in both transverse and longitudinal directions. Then the following considerations could be applied. Let Fig. 52 represent the wall of a fibre seen in surface view and let *ABCD* again be the trace of the index ellipsoid on the surface, *OB* representing the major axis of an ellipse and equivalent to n_γ, the major refractive index of the cellulose, and *OC*, n_α, the minor refractive index. Then, when viewed in transverse section along the direction marked by the full arrow, the effective major refractive index is $n_\gamma^{\perp}$ and in longitudinal section, along the dotted arrow it is $n_\gamma^{\parallel}$. These can clearly be related to n_γ, n_α, and θ by the equations:

$$\frac{(n_\gamma^{\perp})^2 \sin^2\theta}{n_\gamma^{\,2}} + \frac{(n_\gamma^{\perp})^2 \cos^2\theta}{n_\alpha^{\,2}} = 1, \qquad \ldots(2)$$

$$\frac{(n_\gamma^{\parallel})^2 \cos^2\theta}{n_\gamma^{\,2}} + \frac{(n_\gamma^{\parallel})^2 \sin^2\theta}{n_\alpha^{\,2}} = 1. \qquad \ldots(3)$$

If we can measure n_γ and $n_\gamma^{\perp}$ then the only unknowns in these equations are n_γ, n_α and θ. Since n_α is never far from the value 1·530, then effectively we have two simultaneous equations involving only n_γ and θ as unknowns and these can therefore be found.

In practice the determinations are not quite so simple as this, for we cannot observe one and the same cell in both directions. The procedure is therefore as follows. The phase differences of the layers in thin, transverse sections of measured thickness is measured for a large number of cells and the average birefringence calculated. Identical material is then macerated and the refractive indices of the fibres in optical longitudinal section are measured by an immersion method as described earlier (p. 54) (note that this gives a check on the value of n_α). It is found that the values of the major refractive index, $n_\gamma^{\parallel}$, depends on fibre length, so a graph is constructed connecting refractive index with length (this will be discussed in detail later on). Finally, the refractive indices corresponding to the average fibre length are read off from the graph and these are used in the above equations. It must be stressed that these latter refractive indices refer only to the outer layer of the cells, since a Becke line method is employed in determining them. Hence the values of the refractive indices for the outer layer only in transverse section are used, and the calculated values for n_γ and θ refer only to this outer layer. The results are presented in Table IX.

It will immediately be obvious that the structure of the outer layer in bamboo is closely similar to that in sisal and in conifer tracheids, so

TABLE IX

Wall structure in bamboo fibres

Species	*Layers dark in T.S. between crossed Nicols*				*Layers bright between crossed Nicols*		
	Angle, θ, between fibre length and m.e.p. (degrees)		*Cot θ*	*Fibre Length (mm.)*	*Angle, α, between fibre length and m.e.p.* (degrees)*		
	Range	*Average*			*Layer* 1	*Layer* 2	*Layer* 3
Dendrocalamus longispathus	2·0–8·4	5·00±0·24†	11·43	3·04±0·11†	34·0±1·5†	19·0±2·0†	10·0±2·0†
Bambusa arundinacea	2·0–7·5	5·40±0·26	10·55	2·86±0·09	—	—	—
Dendrocalamus strictus	3·0–7·3	6·44±0·34	8·91	2·74±0·10	34·5±4·0	20·5±5·5	12·0±2·0
Melocanna bambusoide	6·5–14·0	10·40±0·38	5·45	1·83±0·07	—	—	—

* Layer 1 is the outermost bright layer; layer 2 is a central bright layer; layer 3 is the innermost bright layer.
All angles calculated from transverse birefringence.
† Standard errors.

that we may take it as rather a general rule that the outer layer of elongated fibrous cells is wound with a molecular spiral of angle about 40° or so. Further, and giving some considerable confidence in this calculated value, we see that the value of the refractive index n_γ is exactly what has actually been observed in other cells. Finally, it may be noted here that unlike sisal and tracheids, bamboo fibres possess a whole series of lamellae which are bright, not just an outer and an inner one. This is illustrated in Plate VIII, Fig. 3. It is naturally impossible to be precise regarding the organization of these intermediate bright layers but, by measuring the birefringence in transverse section and assuming the values of n_γ and n_α are the same as those calculated for the outer bright layer, then the spiral angle can be calculated for each layer from equation (2), p. 138. The corresponding values are included in Table IX. The spiral in the bright layers therefore apparently becomes steeper as the lumen is approached.

By analogy with the fibres of wood and of sisal, we may conclude that the extensive dark layers observable in the transverse sections of bamboo are composed of cellulose chains lying in a much steeper spiral, and for exactly the same reasons. This is, in fact, shown by the X-ray diagram of fibres taken with the beam normal to the length of the fibres which is not included here since it resembles very closely that of hemp fibres (Plate II, Fig. 3) except that it is not so perfect. The m.e.p. of the whole wall is also in harmony with such a view (Table VI). Again there is the peculiarity that the bright layers are not apparently recorded in the X-ray diagram, and this is now so general a phenomenon that it merits some little attention.

We saw in conifer tracheids and wood fibres that the cellulose micelles in the outer layer had very considerable angular dispersion and, possibly, low cellulose content. If we can take the case of sisal fibres as analogous to that of the wood cells, then a tentative explanation of the phenomenon might be attempted. In sisal, the cellulose content of the outer layer in immature tissues is not markedly lower than in mature ones, but it is to be remembered that from the present point of view we are interested in the outer layer in *mature fibres* for which we have no data. It is clearly possible that intensive lignification, involving as it almost certainly does a "swelling" of the cellulose matrix by deposition of the lignin in "intermicellar" spaces, would induce a higher angular dispersion.

There is in the literature a good deal of evidence that lignification induces swelling in cell walls and we may perhaps note one particular case closely analogous to the present one. In jute and hemp fibres it

now seems reasonably certain that the wall structure is essentially the same as in the fibres described here and, fortunately, the course of lignification has been followed rather carefully. It has been observed that the thickness of the outer layer in the wall increases very considerably as lignification proceeds (49) (Table X). This can hardly be

TABLE X

Increase in thickness of the outer layer in the walls of jute fibres during the deposition of inner layers

Fibre type	*Condition*	*Thickness*		
		*Whole wall**	*Inner layer†*	*Outer layer‡*
Protophloem	Young; partially thickened	1·38±0·05	1·19±0·04	0·18±0·04
	Mature; fully thickened	4·30±0·09	3·87±0·09	0·42±0·04
Metaphloem	Young; partially thickened	2·12±0·07	1·68±0·11	0·44±0·05
	Mature; fully thickened	3·81±0·16	3·22±0·16	0·60±0·10

* Under the ordinary microscope.
† Under the polarizing microscope (dark).
‡ Under the polarizing microscope (bright).

due to deposition of new cellulose within the outer layer, since at the time when this increase in thickness occurs the central layer is already present, so that the protoplasm is far removed from the outer layer. It seems far more probable that the development of lignin from some precursor already present involves a "swelling". That this swelling would lead to an increase in angular dispersion is indicated by some observations on the highly lignified coconut fibres. When lignin is removed from these fibres, then the angular dispersion decreases considerably (Plate IX, Fig. 1). It follows as a natural corollary that as the developing fibre lignified, the angular dispersion increased.

Such dispersion would then materially reduce the contribution the outer layer would make to the diagram; and remembering that the chains here are in a flat spiral anyway, so that the arcs are already widely spread, this might cause a virtual disappearance of the diagram of the outer layer except as a contribution to the circular "halo" which accompanies the arcs in all these imperfectly crystalline bodies. This explanation must be accepted with some reserve at the moment, since the sisal fibres used here had been retted, so that we have no information as regards the lignin content in the naturally occurring fibre, and

because in bamboo there is no evidence of the dispersion or low cellulose content needed to make the explanation hold.

It may therefore be concluded in general that the structure of these elongated cells shows a crossed fibrillar organization somewhat like that already found in some algae. We shall see in the next chapter that the resemblance goes even further than this, so that it is salutary to note here at least one major difference, a difference in the extent to which the crossed fibrillar structure goes. In these elongated cells, the layers with different directions are microscopically visible and therefore few in number. In the algae, however, they are submicroscopic in thickness and therefore many in number. Whether this is a really fundamental difference or not it is impossible to say at the moment. We might perhaps in passing note the different rates of development of the two types of cell. In the algae each cell continues apparently to deposit cellulose from the time it appears throughout the whole growing season—and perhaps for more than one year in *Valonia*. With conifer tracheids, a count of the cells developed over a week or two suggests that a tracheid is completely developed within two days from its first differentiation from the cambium. It is therefore a most interesting, if dangerous, speculation that wall layering of this kind in the algae has been associated with changes in the environment, where the cell grows over a long succession of days and nights, while a conifer tracheid grows only for about two days and one night (or two nights and one day).

Structure in other mature cell types

While interest has centred largely around the elongated lignified elements of plants dealt with thus far, observations have naturally been made also on other cell types and we may perhaps glance at one or two of these now. There is little that can be said of the majority of parenchyma cells. The walls are commonly almost isotropic in face view, and whether this means that the cellulose micelles are arranged completely at random, or whether the wall is built up of submicroscopic layers each with its own chain direction rather like minute *Valonia* cells, is not known. In certain cases, however, the structure of parenchyma cells has been worked out in some detail. The cells of coleoptiles in particular have been the object of many investigations. The m.e.p. has been stated to follow a rather flat spiral (50(*a*)) and to show a relation to cell length very roughly as described later. This observation may, however, need to be re-examined in view of more recent work in the electron microscope (50(*c*)) which seems to show the presence in the wall

of two layers, one in which the microfibrils are arranged almost transversely and the other almost longitudinally. Both sets of microfibrils show marked angular dispersion. Similarly the walls of sieve tubes are almost isotropic, and again little is known concerning them though recent electron micrographs are rather illuminating (8). Two cell types do, however, show structural orientations of an interesting type—the vessels, and the cells of the collenchyma.

Vessel elements

The walls of vessel elements are commonly heavily pitted and this naturally leads to such a disturbance of structure that the wall becomes very complicated indeed. The cellulose micelles in all pits, whether the large simple pits of parenchyma cells, the bordered pits of tracheids or the slit pits of fibres, tend to lie parallel to the nearest edge of the pit. This is shown most strikingly in the heavily bordered pits of tracheids (Plate III, Fig. 4) where the border shows a clear Maltese cross. Nevertheless it can be said that in vessels the micelles of cellulose lie almost transversely, though there are some exceptions (50(*b*)). This has been shown both by examination under the polarizing microscope of wall areas free from pits, and by the X-ray photograph of single vessel walls (Plate VIII, Fig. 4). The general run of the cellulose chains can, indeed, be seen in isolated vessels by the run of the mouths of slit pits on those parts of the walls which have been in contact with elongated cells. Thus in Fig. 53 are given a few examples of vessels in which the run of these slit pits is marked by a series of short lines. It will be obvious that while in general the pits lie transversely there are exceptions. The wall structure is subject to sudden and abrupt changes which may perhaps be associated with the very large diameter of these cells.

In transverse section under the polarizing microscope, the wall of vessels is often homogeneous, or very nearly so, and it may be concluded that in these vessels, therefore, the wall is homogeneous in cellulose chain direction. In some cases, however, notably in some vessels of *Fraxinus americana*, almost all vessels in *Sassafras officinale* and vessels of *Castanea dentata* a lamellation can be observed (50(*b*)) which recalls strongly the layering in bamboo fibres and presumably has the same origin. This can be confirmed in *Fraxinus* since those vessels showing this optical heterogeneity also show slit pits whose mouths twist in the wall—the so-called "spiral" pits. These vessels also show, where the vessel element is in contact with another vessel, a series of scalariform pits whose mouths lie transversely, breaking up nearer the lumen into a number of slit pits with tilted slit mouths. All this suggests

very strongly a variation in wall structure very much as recorded above for the fibrous cells. It is a most interesting and peculiar fact that in *Fraxinus* these "anomalous" elements are confined to relatively few

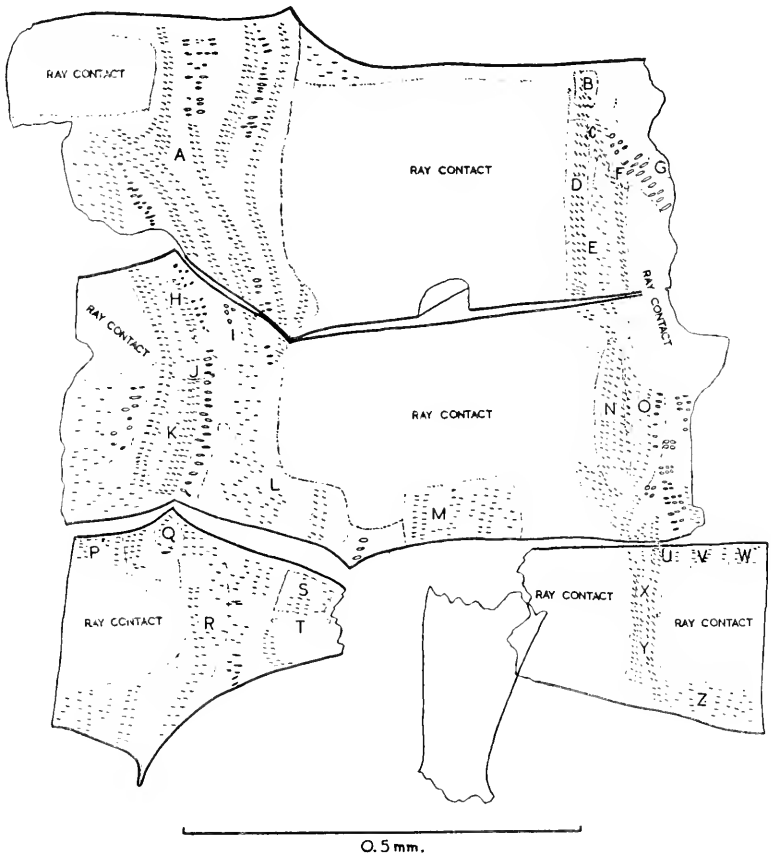


FIG. 53. Three contiguous elements in a vessel of *Quercus alba* cut open and laid out flat. The slit pits mark the directions of the cellulose chains. The angles (in degrees) to the transverse in the areas marked are as follows. A positive sign indicates an incline upwards to the right and a negative sign to the left.

A, −12	H, + 4	O, −20	V, +15
B, −20	I, −10	P, +12	W, +12
C, −43	J, − 4	Q, −34	X, −21
D, −39	K, + 4	R, − 3	Y, −40
E, −29	L, −10	S, − 4	Z, − 7
F, −17	M, 0	T, +10	
G, −45	N, −33	U, +10	

N.B. The contacts to ray tissue are so heavily covered with large simple pits that the wall structure is completely disordered and is not therefore recorded.

vessels; if one element in a vessel is heterogeneous, then all the elements in the vessel are similarly heterogenous; if one element is homogeneous, then so are all the rest.

Collenchyma cells

These cells, occurring in the outer reaches of the cortex in many plants, are often similar in shape to the fibrous cells already dealt with. They differ from these, however, in two respects at least. Firstly, the wall is not uniformly thick, and this is the feature by which these cells may be recognized. In the differentiation of the cells, the walls first begin to thicken in the neighbourhood of the intercellular spaces lying between three or more neighbouring cells. This causes the cells in transverse section to appear thickened at the corners; the thickening may then spread to cover the whole of the walls lying tangentially in the stem—but never to the radial walls—and collenchyma cells have been classified somewhat arbitrarily into four main types according to the pattern of this thickening. In longitudinal view the thickenings can be seen as "bars" running down the length of the cells. Secondly, the wall does not contain lignin, but has a high percentage of pectic substances. It is apparently to this high pectin content that the walls owe their extensibility, and it is certainly the presence of pectin which confers upon the wall its relatively enormous swelling in water. Collenchymatous tissue dehydrated and mounted in balsam is often difficult to distinguish because the thickening bars have shrunk to about the same thickness as the rest of the (unthickened) wall. When, however, the material is replaced in water, the bars swell again to the dimensions observed in fresh material, the swelling being of the order of 150% or more (Table XI).

This swelling occurs, however, only in the transverse plane and, in fact, only in the radial direction. Swelling in length is negligible (Table XIA), a fact which, as first pointed out by Haberlandt (before the structure of cellulose was known), would indicate that the units of structure in the wall lie longitudinally.

The wall structure of the three main types of collenchyma have been investigated (36), and the relevant results for two of them are presented in Table XII. It will be clear that in the main the cellulose chain orientation resembles that in the central layer of very long tracheids or fibres, confirming the suggestion of Haberlandt. In transverse section the walls do show some differences from these, however, when viewed between crossed Nicols. The thickened regions of the walls are usually very clearly lamellated with lamellae alternately dark and bright, but this

TABLE XI

Shrinkage and swelling of the wall bars of collenchyma cells of Petasites vulgaris *L. as seen in transverse section*

Cell No.	*Wall thickness (μ)*				
	In alcohol	*In water*	*% increase*	*Replaced in alcohol*	*% increase over original*
1	2·2	4·9	126	2·4	9
2	2·6	6·0	131	2·9	11
3	2·8	5·7	100	2·8	0
4	2·5	5·3	112	2·6	4
5	2·2	5·2	132	2·5	13
6	3·1	6·5	169	3·3	6
7	2·5	5·9	133	2·6	4
8	2·6	6·2	147	2·7	4
9	2·2	5·0	129	2·4	9
10	2·4	5·3	125	2·5	4
Average	$2{\cdot}5_1 \pm 0{\cdot}07$	$5{\cdot}6_0 \pm 0{\cdot}1_6$	$130 \pm 5{\cdot}0$		

TABLE XIA

Length changes in collenchyma cells of Petasites vulgaris *on dehydration and subsequent hydration. Lengths are given in mm.*

Cell No.	*Length in water*	*Length in alcohol*	*% decrease*	*Length on replacing in water*	*% increase*
1	0·463	0·461	0·43	0·464	0·65
2	0·564	0·562	0·35	0·564	0·35
3	0·539	0·535	0·75	0·536	0·18
4	0·420	0·418	0·47	0·420	0·47

TABLE XII

The major extinction position in collenchyma cells. The figure gives the angle in degrees between the direction of the m.e.p. and cell length

Species	*m.e.p.*	
	Range	*Average*
Heracleum sphondylium	0·0°–1·5°	$0{\cdot}6° \pm 0{\cdot}8°$
Petasites vulgaris	0·2°–4·2°	$2{\cdot}0° \pm 0{\cdot}3°$

lamellation is here not due, in the main at least, to an alternation of cellulose chain direction; it resembles much more closely that already reported in algae (p. 95). If the material is subjected to a preliminary swelling and then stained in ruthenium red, then alternate lamellae take up the stain much more intensely than the rest, and it seems very clear, as first pointed out by Anderson, that the pectin is largely, though not, of course, entirely, confined to separate lamellae.

Alternatively, the wall may be stained with iodine and 70% sulphuric acid in order to demonstrate the distribution of cellulose. Here again, in the collenchyma of *Solanum lycopersicum*(36*a*) and of *Heracleum sphondylium* (36(*b*)) fine lamellation can be observed so that there is no doubt here but that the lamellation is of the cellulose-rich pectin-poor, cellulose-poor pectin-rich, type. With *Petasites vulgaris* (36(*c*)) (showing collenchyma of the tubular type where the thickening of the wall round each intercellular space gives the impression of a uniformly thickened *intercellular space*) lamellation has never been observed in cellulose stains. In this type, therefore, it seems that the cellulose is uniformly distributed through the wall while the pectin occurs mostly in separate lamellae within the cellulose matrix. Since the walls of these collenchyma cells shrink on drying and swell on wetting much as do those of other types (Table XI), it seems unsafe to attempt any close connection between pectin content and swelling. The suggestion has been put forward that the presence of pectin is in some way associated with the presence of large intermicellar spaces throughout the cellulose of these cells, and therefore presumably with a high ratio of non-crystalline to crystalline cellulose fraction, and that this accounts for the high degree of swelling.

The other notable difference between the appearance in transverse section of collenchyma cells and the lignified elongated elements is that the outer bright layer is missing. In its place we find a layer which is isotropic both in transverse and longitudinal view, which does not stain in ruthenium red, iodine and sulphuric acid or in Sudan III. This layer, or cuticle as it may perhaps be called, is in fact of unknown composition. The thick central layer within it is, however, faintly birefringent in transverse section and strongly so in longitudinal view, very much like the central layer in tracheids and fibres. The figures given for micelle direction in Table XII undoubtedly refer to this layer, and this is also the layer which is responsible for the X-ray diagram (which strongly resembles that presented in Plate II, Fig. 2 though the arcs are much more diffuse, in harmony with the general picture of wall structure). A tenuous innermost layer is bright in transverse section between

crossed Nicols, and it now seems probable that this is due, to some extent at least, to the presence of cellulose micelles inclined in a spiral less steep than that in the central layer, though this was not the original interpretation.

It is interesting to notice, in view of these differences of structure between collenchyma cells and the other cells we have looked at in this chapter, the very special function which appears to be confined largely to these cells. Normally collenchyma is produced early in the development of the stem and it is generally considered that its chief role is to provide mechanical strength in such a way as to impede longitudinal growth as little as possible. It would seem that this is achieved through the presence of cellulose chains oriented almost longitudinally, giving high ultimate strength in the longitudinal direction, coupled with a high proportion of non-crystalline cellulose which will thus allow considerable extension.

Cotton hairs

Finally, attention must be called to the very important work which has been going on for many years in different laboratories throughout the world, on a variety of plant hairs. In some way these form more suitable material on which to make growth studies in terms of wall structure since they share with the algae the advantage that their growth is less confined by the presence of neighbouring tissues. In point of fact, detailed observations have been made on only two types of hair. These may be exemplified by the staminal hairs of *Tradescantia* studied by Martens and by van Iterson, and cotton hairs which have naturally been the subject of much wider investigation. For reasons of space, attention here will be confined to cotton hairs on account of their greater familiarity and importance. The structure of the other hairs is in essence very similar to that of cotton which, in turn, we shall find in many respects to resemble rather closely that of the other elongated cells we have reviewed here. A valuable summary of the more botanical aspects of the numerous investigations carried out on cotton has recently been presented by Flint (75).

The pioneer investigations were made by W. J. Balls on varieties of cotton grown in Egypt and, although his observations made in Egypt many years ago have naturally been considerably extended, in the main his interpretations still stand. Cotton hairs take the form of very long (up to a few centimetres) threads with thick walls proliferating from the epidermis of the seed. In transverse section the walls show little structure, but if they are stained in a substantive dye and particularly if

swollen in cuprammonium (Schweitzer's reagent) very fine lamellation may be distinguished. Unlike, however, the lamellation we have studied up to now, these lamellae apparently vary little, if any, in cellulose chain orientation or in content of non-cellulosic substances; in fact the secondary wall of cotton hairs consists of remarkably pure cellulose. The interpretation of lamellation most widely accepted is in terms of a variation in porosity, a variation which recalls strongly the interpretation now put upon the layering in the secondary walls of tracheids and fibres. Observation of whole cotton hairs under the microscope, particularly if the hairs are slightly swollen, reveals the presence of fine striations forming a spiral round the wall which, according to the variety of cotton examined and the age and length of the particular hair, makes an angle with cell length somewhere in the range 25° to 45°. One very striking peculiarity here, however, a characteristic which seems confined to cotton hairs, is that the spiral is reversed more or less regularly along the length of the hair, *i.e.* passing along the hair the spiral may first be right-handed (the so-called Z spiral) then left-handed (the so-called S spiral), again right-handed, and so on. It is somewhat difficult to establish any general rule as to the orientation of the cellulose at the points of reversal; according to published descriptions the "fibrils" may turn to become parallel to the length of the hair at the reversal points or, again, the fibrils of each neighbouring spiral may intermingle with little sign of change in orientation.

Corresponding to this general picture, the X-ray diagram shows the diffraction arcs characteristic of cellulose, spread into arcs corresponding to this spiral arrangement. It should be noted that right- and left-handed spirals give the same X-ray diagram so that no peculiarities in the diagram, due to the reversals of the spiral, are to be expected. Normally the lateral arcs are continuous like those of the wood cells illustrated in this book, and this is undoubtedly a reflection of the fact that generally in a bundle of hairs the spiral angle varies rather widely. When a single hair is examined in an X-ray microcamera, however, the lateral arcs each break up into two spots,* as theory predicts (p. 124) and the diagram then in general resembles that in Plate VI, Fig. 4, except that the arcs correspond to native, and not mercerized, cellulose.

Although the X-ray diagram thus corresponds roughly to only one set of spirally arranged chains in the wall, it is now quite clear that cotton fits in with other elongated cells of the higher plant in possessing wall lamellae whose structure is different from that in the bulk of the

* Private communication from Professor W. T. Astbury.

wall. As in these other elongated cells, one of these is the primary wall whose structure will be discussed in a later chapter. In cotton it is particularly easy to characterize this primary wall since it is the only part of the wall which stains intensely with ruthenium red corresponding, it would therefore seem, to the restriction of pectin to this primary wall region. Below this layer, however, there can be distinguished a lamella which Hock, Ramsay and Harris have called the "winding" and which Rollins has interpreted as the inner lamella of the primary wall. The more recent evidence brought forward by Kerr (51), however, makes it rather certain that this is the outermost layer of the secondary wall, an interpretation which is most satisfactory in the light of the known structure of other elongated cells. Thus, the windings can be distinguished even after the primary wall has been removed mechanically; its component cellulose matrix is well oriented (unlike the primary wall, see Chapter IX) in a spiral rather flatter than in the rest of the secondary wall; further the spiral reverses regularly along the length of the hair and, though the reversal points do not correspond with those in the rest of the secondary wall, the absence of reversals in the primary wall would seem to distinguish it rather completely. There seems therefore little doubt but that this "winding" corresponds both in development and structure to the outer layer of the secondary wall as found in, for example, the tracheids. An innermost layer with similar structure is not, however, found in any variety of cotton.

In terms of this similarity of structure between cotton and other elongated cells, and in view of the effect of environment on wall structure in the algae (p. 107) it is very interesting indeed to note the pronounced effect of light on the structure of cotton hairs. As mentioned above, cotton hairs are characteristically finely lamellated when viewed in transverse section under appropriate conditions. Now it was obvious from the earlier work of Balls that there could be a rough correlation between the number of these lamellae and the number of days over which the secondary wall continued to increase in thickness. Such a correlation has been fully confirmed by later workers, notably by Kerr and his collaborators, and it is therefore certain that the lamellation here is associated, directly or indirectly, with some factor in the environment showing regular daily fluctuation. That this factor is in fact light was shown by Anderson and Moore (44) by growing cotton under constant light conditions. The hairs developed under these conditions showed no traces of lamellation and the observation that other cell types in the stem of the plant, removed therefore from the direct influence of external factors of this kind, continue to show wall

lamellations would seem to indicate that the influence of light is rather direct. It should be noted that this is an effect of light on *porosity* in the cell wall and does not involve any major change in structure. It is therefore not strictly analogous to the effects observed in the algae where a net change in orientation is involved.

CHAPTER VIII

Structural Variations in Homologous Cells

Dimensional relationships in tracheids and fibres

THE RATHER precise picture of wall structure in elongated cells described hitherto, nevertheless leaves a good deal to be desired. Among the outstanding features which still await explanation, the remarkably wide variation in spiral angle between individual cells from the same sample calls attention to itself most forcefully. The question arises as to whether the variation is quite random or if something systematic can be described about it. Together with this variation in structure, for instance, goes also a variation in cell length, in cell width and in wall thickness as well as, probably, variations in chemical composition and in features of the cellulose complex other than orientation. Is it possible that the variation in chain orientation is connected with one or more of these coexistent variations?

A glance over the earlier literature, prior to the year 1934, gives immediate signs of a possible correlation particularly with cell dimensions. Up to that time cellulose chain orientation in cell walls had been investigated almost exclusively through observations of the m.e.p. of walls in surface view, with the exception of fibres of which X-ray diagrams had been used in the elucidation of the submicroscopic structure of cellulose. As we have seen, there is always some doubt as to the precise interpretation of m.e.p.s in terms of cellulose chains except when supported by other evidence, and at that time this evidence was lacking. Nevertheless it seemed rather safe to assume that, whenever wide differences in m.e.p.s were observed between cells, then that in itself intimated correspondingly wide divergences in cellulose chain direction. Taking that for granted, then the data in the literature were most suggestive, particularly in the comparison afforded between fibres, tracheids and vessels. In this order, these are long, thin cells; shorter, wider cells; and short, fat cells. As we have seen already, the general impression of structure is that the cellulose chains in fibres lie relatively steeply, often almost longitudinally; in tracheids they lie in spirals which may or may not be very steep; and in vessels they lie almost transversely to cell length. In other words it appears that the

longer a cell has become during development the more nearly do its cellulose chains lie longitudinally. Certainly even at that time there were obvious exceptions, the outstanding example being the cotton hairs. As we have seen, these cells, though extremely long, nevertheless persist in laying down cellulose chains at an angle of some 30° to cell length. Cotton hairs are, however, exceptional in other ways too, the most notable being the frequent reversals of the spiral at intervals along the length of individual hairs, so that it seemed safe to leave such exceptional cases for the time being out of account. In any case, once the possibility was grasped that there might be a correlation between cell length and wall organization then it became clear at once that correlations could only be expected among individual cells of similar type, and that other factors might intervene to render comparisons between cells of different types invalid. We shall, in fact, see that when cotton hairs are considered by themselves they form no exception.

It seemed therefore worth while to investigate the possibility of a connection between cell dimensions and wall structure in a population of cells of similar type, within which the only major differences would be those of cell dimensions. The investigation would obviously call for the observation of some thousands of cells, so that an optical method had to be employed rather than the (considerably more intricate and time consuming) X-ray analysis of single cells. This restricted attention immediately to tissues within which the cells were all of the same type, in order to provide large numbers of similar cells without the necessity of selection, and in which the m.e.p. could be relied upon to give a reasonably accurate idea of chain orientation. For these reasons, and for others which will appear as the argument proceeds, the wood of conifers was chosen. With the exception of ray tissues, wood of this kind consists only of tracheids all of which have been developed in the same way, from identical cells, and have, if not identical, at least very similar chemical composition. There are, in addition, features of development in these woody stems which make the selection of length classes among the cells very easy indeed.

The results of the investigation thus initiated, covering the examination of some 60,000 individual tracheids, first appeared in 1934 (47(*c*)), and it at once became evident that a relation of the type suspected did in fact obtain. Although these earliest results led to an error in interpretation, it is nevertheless interesting to follow their development into the later generalizations to cover cells of other types and into the more modern interpretation of the correlations thus revealed. In order to obtain a clear insight into these matters we must first make a small

diversion into the realms of ontogeny to see how these tracheids are developed.

The development of conifer tracheids

If a piece of bark is removed from the trunk of a conifer tree during spring, and it is particularly easy to remove the bark only at this time,

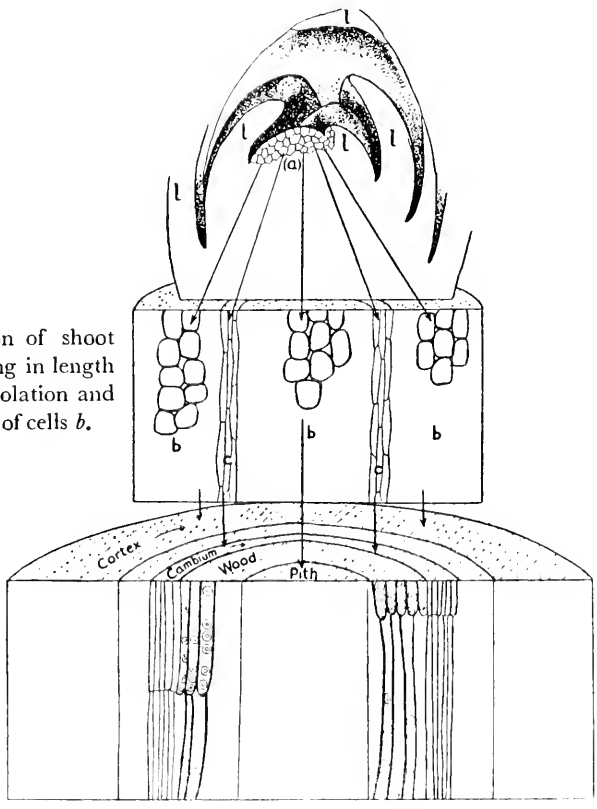


FIG. 54(*a*). Diagrammatic representation of the location of the cambium. The cells are drawn many times too large for clearness of figure.

then it will be found possible to remove from the surface of the wood a thin film with the consistency of a weak jelly. Examination of this under the microscope will reveal that it is actually a cellular tissue consisting of rather uniform elongated cells with very thin cell walls.

Provided that such material is collected early enough in the growing season, then it is found that all the cells are of this kind, and have densely cytoplasmic contents without the obvious vacuoles which will appear later in derived cells. This layer then constitutes cells newly cut off from the *cambium*, the tenuous living layer of the stem, which produces during each growing season the phloem on the outside and the xylem, or wood, on the inside, and is therefore responsible for the growth in thickness of the stem year by year. Towards the end of each season this cambium ceases its activity and stiffens in consistency, presumably due largely to a lowering of the water content, and from then on until the onset of the next growing season the bark, cambium and xylem adhere together very firmly. During spring, however, cambial activity is again renewed, so that year by year this tenuous living layer lays down a new cylinder of wood surrounding the old, and this continues throughout the life of the tree. These yearly increments of wood cause the appearance on the cross-section of a trunk of the so-called *annual rings*.

The production of wood tracheids is in some ways a most remarkable business. The cells of the cambium are quite uniform in shape, taking the form of long, thin cells, much narrower in the radial direction than in the tangential, and with six longitudinal faces much as described and figured in previous pages (Chapter II) (Fig. 54(*a*)). These cells are growing, increasing largely in radial dimensions (but, as we shall see, also in length) by the continuous production of new protoplasm until, when some undefined size has been attained, they divide into two cells. This division is peculiar, however, in that the division wall does not lie transversely, cutting the cell into two of the same width but one-half the length. Instead, it lies longitudinally, cutting the cell into two of the same length but of only one-half the width. Further, this dividing wall always lies tangentially to the stem so that the two daughter cells lie along a radius and never along a tangent to the stem. The occasional transverse divisions (or pseudo-transverse since the dividing wall is never really horizontal) are too few to be of account here; and in any case such a dividing wall rapidly swings round to become a new radial wall, by a mechanism which is not understood. The divisions with which we are most concerned, therefore, are longitudinal tangential. Now when such a division occurs on the inner face of the cambial cylinder, the two daughter cells (Fig. 54(*b*)), commonly behave in a dissimilar manner. We shall consider here only the case in which the outer of them remains a cambial cell. This continues to grow as before until a further division ensues. The one facing the wood, however, increases in radial dimensions to a much greater extent; during the

early period in the season, when water is plentiful, it may increase up to four or five times the normal radial width of a cambial cell, reaching a size of some 20 μ or more and equal to, or even greater than, the tangential dimensions (Fig. 54(*b*)). Increase in size is rather sudden, occupying at most only a few hours, and involves the intake of relatively

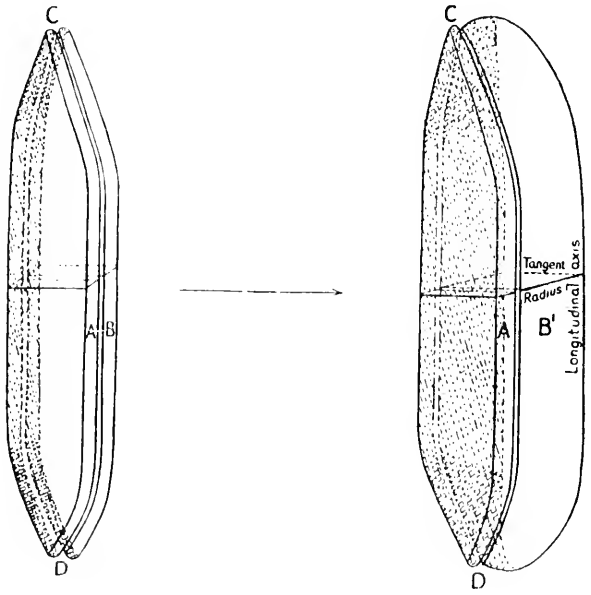


FIG. 54(*b*). Diagrammatic representation of two daughter cells in the cambium, one of them (*B*) becoming differentiated as a tracheid (*B'*). The tracheid appears here blunt ended; this is a consequence of the reduction of length in the drawing.

large quantities of water. The increase in size is, in fact, due largely to the development of a vacuole and the process is therefore called *vacuolation*. All this time the wall surrounding the cell remains thin; it is the primary wall whose structure and behaviour we shall examine later on. Once the differentiating cell has reached its full size, however, the wall begins to thicken by the deposition of a series of layers collectively called the secondary wall with which we are still concerned, and these finally take the form which we have investigated in the last chapter.

This process apparently goes through to completion once in about every two days throughout the season, and leads to the production of radial files of tracheids equal in number to the cells in one tangential layer in the cambial region, every cell in one file having developed from one and the same cambial cell. This provides us with a remarkably uniform tissue. Towards the end of the season, the radial expansion on differentiation becomes less and less so that the tracheids become radially thinner while retaining the same tangential dimension,* and at the same time the walls become thicker. It is tracheids of this latter type which we have been considering up to now. For the moment we will here confine our attention to the larger tracheids in the early wood for a very simple reason. It will be evident from the above discussion that there might be a distinct difference between radial and tangential walls in tracheids in virtue of their different associations with development in the cambium; the radial walls are continually stretched laterally during both differentiation and during the growth back to normal size of the cambial initials after a division, while the tangential walls are new at each division (and therefore in each tracheid) and do not undergo much lateral extension. Now in the late wood, not only are the radial walls difficult to observe on account of their narrowness relative to the tangential walls (isolated cells lying therefore on their tangential and only seldom on their radial walls), but it is difficult to distinguish them from tangential walls since the only difference is in width. This difference cannot be used here since, as will be seen later, all the walls other than the one being observed are, of necessity, removed. In the spring wood tracheids, however, none of these objections apply. The cells are just as liable to lie on their tangential as on their radial faces, and these can be distinguished since only the latter are pitted. This absence of pits on tangential walls is, in fact, rather general among tracheids except in the last few cell layers of the late wood, and it is this exceptional case which makes it particularly difficult to use late wood tracheids for the present observations.

Now while this process is going on, the cambial cells are continually elongating. This change in dimension is much slower than the lateral changes associated with the longitudinal tangential division, but nevertheless reaches in time very considerable proportions so that the cells may double their length in some thirty years (see *e.g.* Table XIII). It therefore follows that, since the cambial cells are continually cutting off "replicas" of themselves, then tracheids in the inner annual rings of

* In fact becoming progressively somewhat wider, a minor change in dimension which will not be considered here.

wood are shorter than those in outer rings, and there is normally a progressive increase in tracheid length from inner to outer rings.

This gives us a ready means of settling the possibility of a connection between cell length and wall structure in these cells.

Variation of spiral angle with tracheid length

The earlier investigation consisted of a series of measurements of the m.e.p. of tracheids taken from various annual rings at the same level in a variety of conifer trees, the determinations being made using the

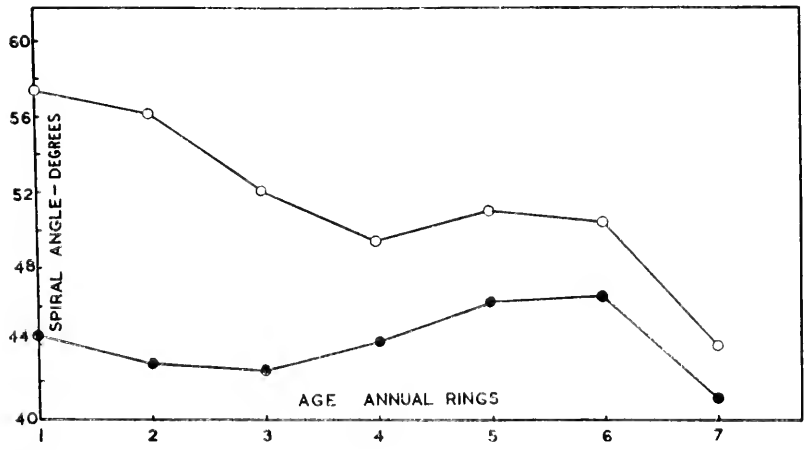


FIG. 55. Graphical representation of the variation of the average spiral angle across the annual rings in *Cedrus*.

—●— tangential walls.
—○— radial walls.

The angle given is that between the m.e.p. and cell length.

technique described in the last chapter (p. 116). Parallel with this, a series of length measurements were made on comparable pieces of wood so that the average tracheid length and the average spiral angle was available for each annual ring. In view of the above description of the development of tracheids, it will be clear that radial and tangential walls had to be considered separately and this separation is maintained in Fig. 55 and Table XIII, where a representative set of data is provided. It is abundantly clear that the relation between cell length and spiral angle is of the type expected. At the time this work was published, it was thought that the m.e.p. referred to the whole wall thickness, but

TABLE XIII

The relation between the average length of conifer tracheids in an annual ring and the cotangent of the spiral angle

Species	*Annual ring*	*Inclination θ on radial walls (degrees)*			*Length in μ*		$\frac{L}{\cot\theta}=K_n$
		Variation	*Average*	*cot θ (Average)*	*Variation*	*Average*	
Cedar	2	36·0–67·0	56·0±1·0	0·687±0·030	729–1560	1071±27	1558±78
	3	39·8–71·0	51·9±0·9	0·796±0·78	747–1700	1200±33	1507±64
	4	36·6–70·0	49·3±1·1	0·891±0·036	916–1880	1358±36	1524±73
	5	36·4–69·2	50·9±1·0	0·840±0·032	955–1760	1310±24	1557±66
	6	37·0–62·2	50·4±1·0	0·850±0·027	975–1720	1326±35	1558±64
Japanese Larch A	1	40·0–71·0	56·0±1·0	0·697±0·026	394–1320	728±32	1045±59
	2	36·0–73·0	53·3±1·2	0·759±0·038	339–1269	784±33	1034±67
	3	35·8–65·6	52·0±1·1	0·780±0·032	567–1210	850±26	1090±55
	4	35·0–69·4	50·8±1·2	0·848±0·035	434–1375	873±33	1018±52
	5	37·6–63·0	49·1±0·9	0·899±0·029	496–1374	931±26	1036±44
	6	34·0–58·4	45·5±0·7	0·997±0·027	459–1618	1034±44	1036±53
	7	34·8–54·6	44·0±0·8	0·987±0·027	620–1812	1094±36	1110±47
Japanese Larch B	1	32·0–71·0	51·3±1·5	0·855±0·044	471–1164	800±22	935±57
	2	37·8–79·5	57·5±1·1	0·650±0·032	462–1140	768±24	1182±69
	3	34·0–73·0	48·8±1·5	0·903±0·041	484–1212	800±24	886±46
	4	38·2–66·6	48·1±0·9	0·928±0·033	483–1204	868±29	935±44
	5	35·6–58·0	49·1±0·7	0·880±0·024	415–1276	889±30	1010±44
	6	31·4–62·0	44·8±1·1	1·039±0·039	532–1470	970±32	933±46
	7	30·0–58·8	43·0±1·1	1·113±0·041	611–1800	1028±33	923±45
Abies nobilis	2	42·8–68·8	53·3±1·0	0·746±0·024	754–2190	1467±39	1967± 81
	3	42·4–65·0	51·9±0·8	0·791±0·023	862–2540	1799±65	2270±104
	4	34·8–64·8	47·9±1·0	0·920±0·032	1130–2540	1925±49	2090± 98
	5	39·2–64·6	50·7±0·8	0·831±0·025	1462–2794	2205±47	2650± 66
	6	35·8–62·4	49·8±0·9	0·873±0·027	1488–2712	2165±40	2483± 81
	7	36·0–64·0	48·8±1·0	0·956±0·035	1485–3520	2408±61	2522±110
	8	30·0–52·8	44·7±0·9	1·055±0·034	1818–3510	2420±53	2292± 89
	9	30·8–60·0	44·2±1·0	1·056±0·039	1560–2980	2424±39	2300± 92
	11	23·0–47·6	34·7±0·8	1·495±0·046	1440–3699	2666±68	1782± 71

now we know that in fact it corresponds rather closely to the chain direction in the central layer only of the wall.

The steepening of the spiral is always much more marked in the radial walls than in the tangential walls and this, taken with the fact that there is a reasonably close parallel between the average length and the average cotangent of the spiral angle (Table XIII) led to the suggestion that the relation between spiral angle and length is of the form

$$L = K \,.\, \cot \theta$$

for the radial walls only. This is the relation to be expected of a spiral elongating at constant girth and it was in these terms that an interpretation of this relation was first attempted. Since the only cells which elongate here are the cambial cells, this involved the assumption that the primary wall of these cells contained cellulose chains oriented in directions similar to those in the tracheids which they produced. Now that we know this to be untrue (p. 177) this tentative interpretation falls to the ground. There was in any case always the difficulty that, if the elongation of the cambial cells causes a steepening of the spiral then, the radial expansion should cause a much more pronounced flattening unless other collateral assumptions are made.

That the steepening of the spiral in passing from inner to outer annual rings is nevertheless a real one, and not associated with any peculiarities involved in the determination of the m.e.p., is shown most effectively by the X-ray diagram of wood. A series of three such diagrams is illustrated in Plate V, Fig. 3, and it will be clear by comparison of these diagrams with those of the model spirals in Plate VI, Fig. 1, that a steepening of the spiral does in fact occur. This has now been verified abundantly with many species both in the author's laboratory and elsewhere. There is therefore no doubt as to the reality of the phenomenon.

In seeking an alternative explanation it was decided to investigate the possibility that a relation of this kind existed, not only among tracheids cut off from the same cambial cell but also among tracheids generally in a tree irrespective of the cambial cell from which they were derived. The determinations were therefore repeated on a series of tracheids chosen at random from a piece of wood. Now, however, that it was necessary to determine chain direction and length in one and the same cell it was no longer possible to make use of the m.e.p. Instead, tracheids were chosen showing striations, which we now know to correspond in direction to that of the cellulose chains in the central layer, and attention was confined therefore to late wood tracheids where this layer is well

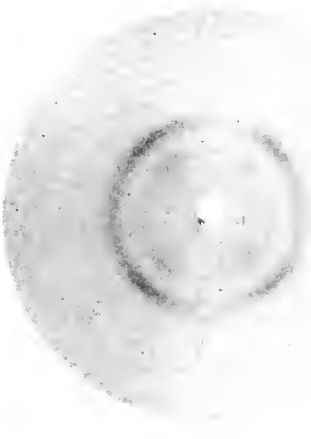


FIG. 1. X-ray diagram of immature sisal fibres (outer layer only of secondary wall). Note that the general disposition of the arcs resembles that in Plate VI, fig. 4, indicating the presence of flat spirals.

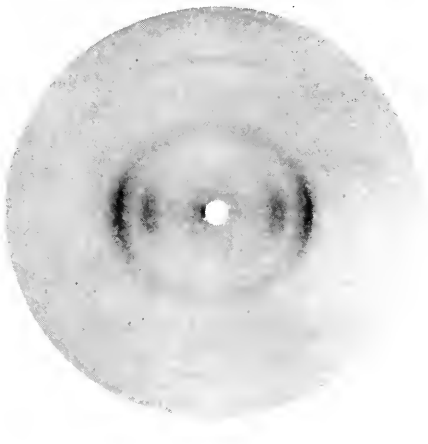


FIG. 2. X-ray diagram of mature sisal fibres (outer and inner layers both present). The diagram shows the presence of steep spirals only—corresponding to the structure of the inner layer—with no definite sign of the flatter spirals which should still be present in the outer layer.

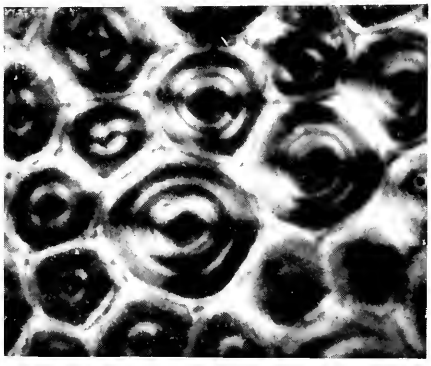


FIG. 3. Transverse section of bamboo fibres between crossed Nicols. Note the alternation of bright and dark lamellae.

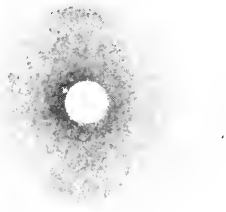
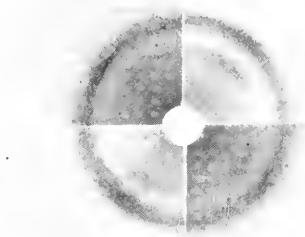


FIG. 4. Enlargement of an X-ray micro-photograph of the (single) wall of a vessel element of *Quercus* (oak) (length of vessel parallel to longer edge of page). The diagram consists of two arcs corresponding to spacings 3·9 Å., lying to the top and bottom of the diagram. The cellulose chains in the wall therefore lie almost transversely.

[*Facing p.* 160



Note that delignification has had at least two effects: (1) the background is less intense, corresponding to the removal of the scatter from lignin; (2) the cellulose arcs are rather narrower tangentially, corresponding to a decrease in angular dispersion. There is also a suggestion that the arcs are slightly narrower radially, which would suggest a slight increase in crystallinity, *e.g.* possibly an increase in micelle size.

FIG. 1. Sector diagram of Coir before and after delignification.
(See Plate II, fig. 3, for details.)
Upper left and lower right: untreated.
Upper right and lower left: delignified.

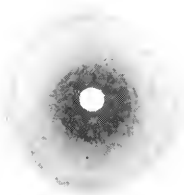


FIG. 2. X-ray diagram of a block consisting of parallel strips of cambial tissues, beam perpendicular to flattened face of strips (and therefore in effect along a radius to the trunk surface), direction A, fig. 59. Note that the arcs are almost circular but have a higher intensity towards the top and the bottom. This indicates a preference in the specimen for transverse orientation of the cellulose chains.

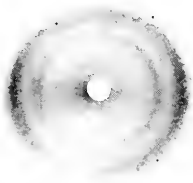


FIG. 3. As in fig. 2, but beam parallel to the flat surfaces and perpendicular to cell length (direction B, fig. 59).

developed. A selection of the results is presented in Table XIV, chosen from a more abundant set of data (47(*e*)). It will be noted that the angle in the table is called the "standard" angle. This refers to the fact

TABLE XIV

The standard angle $\theta_{1\cdot00}$ for tracheids of known length
(the unit of breadth is 1 unit=24 μ,
and
for length 1 unit=74 μ)
selected at random from a much larger body of data.

Species	a	b	c	d	e	a	b	c	d	e
Picea sitchensis	2	11·7	1·25	34·6	37·7	10	23·1	1·70	16·6	19·8
	4	13·6	0·66	38·6	22·8	3	23·5	1·36	11·0	17·0
	4	14·6	0·80	27·6	21·0	4	24·0	1·34	10·8	12·5
	3	15·2	0·96	26·2	21·6	2	24·6	1·89	15·1	23·7
	3	16·4	1·01	22·9	22·7	4	25·0	0·96	13·5	11·4
	10	17·2	1·05	18·2	19·0	3	25·6	1·07	12·8	13·3
	4	17·9	1·05	17·0	16·7	10	26·1	1·66	11·6	17·6
	2	19·1	1·29	16·1	20·0	10	27·6	1·46	12·3	16·4
	4	19·8	1·37	17·4	24·3	4	28·0	1·04	10·7	10·7
	2	20·2	0·98	15·6	15·8	3	28·5	1·22	12·2	14·5
	4	20·8	1·26	11·2	12·9	10	29·2	1·33	11·7	12·4
	3	21·6	1·06	18·1	17·6	3	29·8	1·00	12·5	12·0
	2	21·9	1·25	16·2	19·1	10	35·9	1·18	8·8	10·3
	4	22·0	1·78	15·5	20·0	10	37·8	1·43	8·4	10·6
Abies nobilis	3	13·0	1·44	36·5	58·8	3	24·0	1·90	19·5	31·5
	3	14·0	1·14	37·3	43·2	3	25·0	1·20	19·1	22·8
	3	15·2	1·61	28·5	47·0	10	25·2	1·01	22·3	18·5
	3	15·5	1·55	30·0	54·3	3	26·2	0·82	23·8	18·2
	3	16·1	1·34	33·2	47·0	3	26·6	1·72	20·5	37·0
	3	18·4	1·92	23·2	49·2	10	27·0	1·48	14·2	19·7
	3	19·5	1·60	32·4	58·2	10	29·6	1·06	12·6	13·1
	3	19·9	1·61	25·1	38·9	10	29·6	1·13	19·9	17·8
	3	20·2	1·51	24·6	39·0	10	37·4	1·80	12·8	23·6
	3	21·3	1·03	23·8	21·5	10	41·0	1·63	13·1	19·4
	3	23·8	1·69	21·0	42·0					

a=Annual ring from which tracheid is chosen.
b=Length of tracheid.
c=Breadth of tracheid.
d=$\theta_{1\cdot00}$
e=Average θ.

that in any tracheid the angle varies along the length of a cell so that the spiral becomes steeper towards the tips. Fortunately the angle θ at any point varies with the breadth b of the cell at that point in such a way that $b/\sin\theta$ is roughly constant. It is therefore possible to calculate for any tracheid the value which θ would have if the tracheid were of some standard breadth not far removed from the real breadth. The standard

breadth chosen was 24 μ and it is in these terms that the standard angle is defined.

It is abundantly clear from Fig. 56 that the length of these tracheids

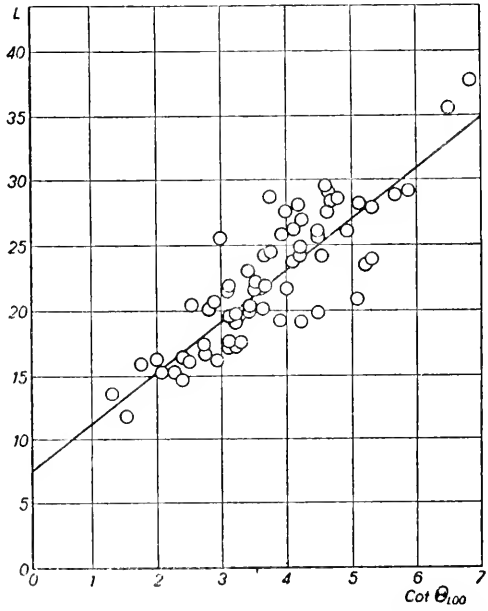


FIG. 56. The relation between cell length and spiral angle in individual tracheids of *Picea*.

is closely correlated with the cotangent of this standard angle. The relation can obviously be fitted to an equation of the general type

$$L = A + B \cot \theta_{1\cdot00},$$

where A and B are constants or, if the breadth b of the tracheids is included,

$$L = A + B\sqrt{(b^2 \operatorname{cosec}^2 \theta - 1)}.$$

The regression line drawn in Fig. 56 represents the equation,

$$L = 568 + 293 \cot \theta_{1\cdot00},$$

where L is in μ, and a similar regression line has also been observed for one other species of conifer wood.

There can thus be no doubt but that among these tracheids, at any rate, there is a rather close relationship between the length of a cell and the structure of the central layer of its wall. It is natural therefore to inquire whether a similar relation holds for the other layers of the secondary wall. This inquiry sets us a more difficult task, and, as yet,

it is possible to give an answer for the outer layer only of the wall, and even that is an incomplete one. The difficulty here is that striations corresponding to the orientation in this layer are seldom observed unless the tracheids are treated somewhat roughly; in which case it is impossible to ensure that the wall structure has not been deformed. Recourse has therefore to be made to more indirect optical methods.

If thin transverse sections of a piece of wood, including the first few annual rings, are observed under a polarizing microscope then it is found that the birefringence of the bright outer layers of the tracheids, as measured by the method described earlier (p. 69), steadily decreases outwards from one annual ring to the next (provided of course that the equivalent regions are compared from each annual ring). Figure 57 gives a good example of this phenomenon. Now, provided that other structural features remain constant, this must mean that the molecular spirals are becoming steeper on passing from the pith outwards. There is at present no reason to suspect any variation other than in orientation so that we have here at any rate qualitative evidence of the length/angle relationship suspected to hold. It is impossible to be precise about the relationship, but a rough approximation may be achieved in the following way. If we assume that the highest birefringence observed in these sections corresponds to quite transverse cellulose chains (and the close correspondence between this maximum figure and that observed in sections cut parallel to the cellulose chains in this layer, see p. 131, suggests that this is not far from being true) then the spiral angle can be calculated for any other value of birefringence at any other point in the section. This has been done and the calculated angles are included in Fig. 57. These are naturally liable to considerable error, but they do show in a striking way that the type of length/angle relationship already found for the central layer holds also for the outer layer. It seems reasonable to expect that a similar statement will eventually be possible for the innermost layer, so that it can be said even now that the chain orientation in the whole wall is conditioned by cell length.

This is a point of very considerable importance in so far as the physical properties of timber depend on chain orientation, but before considering its implications it will be as well to glance, if only rather briefly, at the other cell types where a similar relation is known, or suspected, to exist.

Dimensional relationships in bamboo fibres

While the later part of this work was still in progress the opportunity presented itself of making a somewhat similar series of observations on

the fibres of bamboo (48(*c*)). The methods followed were those outlined in the last chapter (p. 137). X-ray diagrams soon showed conclusively that bamboo species with longer fibres contained also steeper molecular spirals, and mechanical separation of the fibres of one species into length classes, with subsequent reparallelization in collodion, similarly yielded X-ray diagrams from which the same deduction could be made.

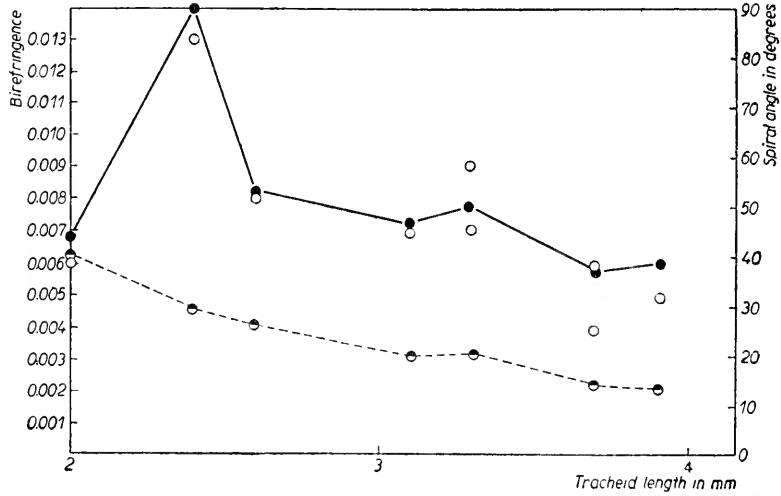


FIG. 57. The birefringence in transverse section of the outer layers of tracheids in *Pseudotsuga*.

○ birefringence. Each point represents the average of 20 observations.
—●— the inclination of the cellulose chain in the outer layers to cell length calculated from the birefringences.
—◓— spiral angle in central layer.

A more exact expression of this relation can be given in terms of the m.e.p. of these cells. As we have already seen (p. 140) the structure of the walls of bamboo fibres is such that the m.e.p. cannot be considered to give a meticulously accurate estimate of chain direction, but again there is good reason to accept it as a close approximation to the chain direction in the thick layers. As shown in Table XV, the m.e.p. is inclined to cell length at an angle of 5–6° except in the case of *Melocanna bambusoide* where the angle is about 10°. This is in good harmony with the X-ray diagrams, and this is one of the lines of evidence which indicates very strongly the close agreement between the m.e.p. of the wall and the chain orientation in those layers which are dark in transverse section between crossed Nicols. It is clear from the table that the variation in m.e.p. is closely paralleled by a variation in cell length.

TABLE XV

Cell length and spiral angle in bamboo fibres

Species	*Angle (θ) between fibre length and m.e.p. (degrees)*			*Fibre length (mm.)*
	Range	*Average*	*Cot θ*	
Dendrocalamus longispathus	2·0– 8·4	5·00±0·24	11·43	3·04±0·11
Bambusa arundinacea	2·0– 7·5	5·40±0·26	10·58	2·86±0·09
D. strictus	3·0– 7·3	6·44±0·34	8·91	2·74±0·10
Melocanna bambusoide	6·5–14·0	10·40±0·38	5·45	1·83±0·07
	*Angle (θ) as determined by the half intensity width of the 002 arcs on the X-ray diagram**			
Bambusa polymorpha		10		3·59
D. longispathus		10		3·04
B. arundinacea		10·5		2·86
D. strictus		10·5		2·74
M. bambusoide		24·5		1·83
D. strictus macerated fibres, selected and reparallelized		11·5		2·0
D. strictus macerated fibres, selected and reparallelized		10·5		3·0
D. strictus macerated fibres, selected and reparallelized		8·5		4·0

* These angles are invariably larger than those defined by the m.e.p. for the same species at the same length. This is because the m.e.p. measures the *net* orientation (with a slight error due to the presence of the thin layers with a different orientation, see p. 117) whereas dispersion about this direction contributes to the spread of the arcs.

This is perhaps more obvious in Fig. 58 where the line drawn through the points (not calculated because the points are so few) represents the relation

$$L = 750 + 200 \cot \theta,$$

where θ is the average inclination to be expected in fibres of average length L (in μ). While the points are obviously far too few for any reliance to be placed on such a quantitative relation, it is nevertheless satisfactory that it takes the form already described for tracheids and the constants are of the same order.

It is interesting next, therefore, to turn again to the outer layers in the secondary wall of these fibres to examine any length relationships there. Thanks to the painstaking attention to this problem paid by Dr. Kartar Singh in my laboratory we are able to be much more certain about the condition here than was possible with the tracheids. We saw in the last chapter that the refractive index of bamboo fibres for light vibrating

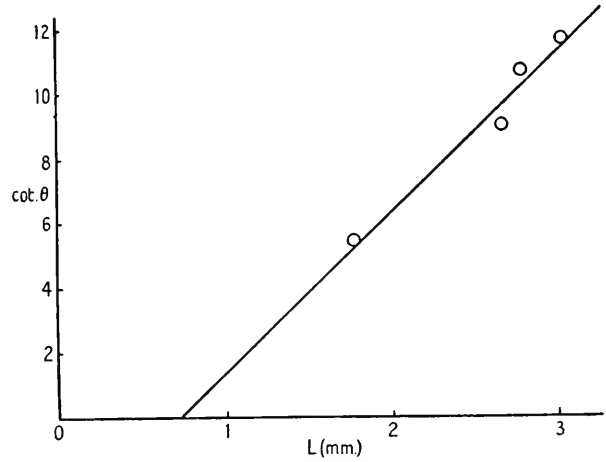


FIG. 58. For explanation, see text.

parallel to their lengths is a function of length. The function can be expressed by saying that this refractive index, $n_\gamma^{\parallel}$, increases linearly as length increases, while the refractive index, $n_a^{\perp}$, for light vibrating perpendicular to the length, decreases linearly as length increases. We saw in the last chapter (p. 138) that by combining optical determinations on whole cells with those made on transverse sections it is possible to calculate, for the outermost layers of these fibres, the specific refractive indices n_γ and n_a of the cellulose and the angle θ between the molecular chains of cellulose and cell length, for fibres of average length. Now taking the calculated value of n_γ as 1·60 we can then estimate, from the value of $n_\gamma^{\parallel}$ for *any* fibre of length l, the value θ_l for the spiral angle in the outer layer. The relation which can then be derived between l and θ_l takes the form

$$l = 5980\ (\cot\theta_l - 0{\cdot}95),$$

so that in these outer layers the variation of θ_l with l is less than with inner layers. It is further interesting to note that, provided the linear regression line can be extrapolated to zero length, then no fibre should have an outer spiral flatter than about $\theta = 45°$.

Confirmation of the presence of flatter spirals in these outer layers comes from the observation that spiral markings can sometimes be seen on these outer walls where inclination to cell length is closely similar to the inclination of the cellulose chains as predicted from the above relation. There can therefore be no doubt but that the observed variation in $n_\gamma^{\parallel}$ with length is largely attributable to a change in the net orientation of cellulose chains. Reorientation alone will not, however, explain the *decrease* in $n_a^{\perp}$ with increasing length; for a moment's reflection will show that simple change in orientation implies a rotation of the index ellipsoid about the n_a direction so that $n_a^{\perp}$ $(=n_a)$ should be invariate. The observed change in n_a must therefore be associated with variation in other subsidiary factors. One possibility is that the angular dispersion of the cellulose chains is less for longer fibres, *i.e.* the "micelles" are more nearly parallel to each other in longer fibres. This would clearly involve a small decrease in $n_a^{\perp}$ coupled with a small increase in $n_\gamma^{\parallel}$. An increase in the crystalline/non-crystalline ratio in longer fibres would also give the same effect and it is therefore perhaps of significance to note that there is some evidence that this may indeed be a factor involved here (48(*a*)). The first observation of importance made was that longer fibres have a higher density (Table XVI). Densities

TABLE XVI

Cell length, refractive indices and wall density in selected length classes of some bamboo fibres

Species	*Length*	*Density*	*Refractive indices of side walls as seen in macerated fibres*		*Crystalline-non-crystalline ratio* (Hermans)
			(*Light vibrating parallel to cell length*)	(*Light vibrating perpendicular to cell length*)	
D. longispathus	3·78±0·13	1·536	1·5801	1·5272	—
	3·15±0·10	1·535	1·5779	1·5278	—
	2·10±0·01	1·532	1·5742	1·5289	—
D. strictus	3·57±0·11	1·535	1·5786	1·5274	58%
	2·35±0·08	1·532	1·5740	1·5286	64%
	1·51±0·08	1·529	1·5710	1·5295	64%

were determined on fibres dried *in vacuo* over phosphorus pentoxide by flotation in mixtures of carbon tetrachloride and nitrobenzene, both bone dry, and the fibres were given a preliminary purification to Cross and Bevan cellulose. The figures for the density refer therefore only to

the cellulose matrix of dried fibres. Now since density here therefore depends on the closeness of packing of the constituent cellulose complex, it will be clear that a higher density, involving a closer packing, implies a higher proportion of crystalline material. There was sufficient encouragement to send samples to Dr. P. H. Hermans of Utrecht, Holland, who has perfected a method of determining the crystalline/non-crystalline cellulose ratio by X-ray examination. The implications of the density determinations are fully justified, as Table XVI will amply bear witness. Unfortunately a second sample gave somewhat discordant results, but there was here clear evidence that the fibres had not been purified quite so thoroughly as had those of the first batch so that perhaps less reliance is to be placed on these later results. Although, therefore, it is not possible at the moment to be very certain about it, there remains the strong possibility that some part of the variation of refractive indices with length may be associated with a change in the amount of crystalline material in the fibre.

Relationships in other fibrous cells

In both conifer tracheids and bamboo fibres, then, there is the clearest possible evidence that the molecular architecture of the secondary walls depends in no small measure on the dimensions of the cells. Somewhat the same situation would appear to obtain also in the other fibrous cells we had occasion to examine in the last chapter although, unfortunately, these have not been worked in sufficient detail to allow the facts to be ascertained with the precision thus far revealed. These other cells can therefore be dealt with very briefly.

In both sisal and cotton the evidence we have refers to the outermost layers only of the secondary walls, but comparison with the data available in conifer tracheids makes it reasonable to assume that a similar relation could be established for all secondary wall layers. We have seen that, in sisal, fibres can readily be obtained with only the outermost layer present, and that such cells yield an X-ray diagram quite different from that of the more mature tissues which carry also the inner wall layers, even though the two groups of cells are not significantly different in length. This we have seen is due to a different orientation in the outer and inner layers. If, now, immature cells of different length are compared, then it becomes quite clear that the spiral in shorter cells is flatter than it is in longer cells. The observations are too few to attempt any quantitative relationships, but it is satisfactory to note that the figures would be in general harmony with the quantitative results presented above for tracheids and bamboo fibres.

Our present knowledge of cotton is in somewhat the same position. We owe the relevant data to the careful determination by Meredith, at the British Cotton Industries Research Association at Didsbury, Manchester, of the refractive indices of large numbers of cotton fibres of various lengths. The results, therefore, are quite comparable with those on the outer layers in bamboo and show very similar phenomena. Again, therefore, it is clear that, even in this peculiar type of cell with its reversals of spiral sign, there is nevertheless the same kind of connection between cell length and wall architecture. Certainly the spiral in cotton is much flatter than we might have expected it to be in terms of the enormous length of the cells, but this might easily be associated with the reversals in sign. In fact, as far as the evidence goes at present, it might be possible from the present point of view to consider cotton hairs as a series of comparatively short cells arranged in a filament.

Finally, brief mention may be made of monocotyledonous fibres, other than bamboo, worked by Meeuse. Here we have available no quantitative determinations at all, but Meeuse does make the remark that there is a tendency for longer fibres to possess steeper wall spirals.

Although quantitative relationships are therefore lacking except in two cases, it seems at the moment very probable that the connection between cell length and molecular architecture may be quite a general one. The lack of a mathematical expression in three of the cases cited here cannot be considered of much consequence, for the relationships quoted for tracheids and bamboo fibres have no theoretical foundation and must be regarded somewhat in the same way as are mathematical expressions of growth rates, as convenient summaries of data with no obvious fundamental significance. This is not to say, of course, that the phenomenon itself is of no significance, just as it would be nonsense to deny significance to the shape of a growth curve even though the mathematical formulation of the curve has no fundamental value. On the contrary, the connection between cell length and spiral organization must reflect something very fundamental indeed in the cell mechanism. What this something may be we can hardly hazard a guess just now, and it is perhaps better to leave this chapter as a bare record of the phenomenon. Indeed, we can hardly begin even to think about the implications without paying first of all some considerable attention to the conditions obtaining during the growth of the cell. It will therefore be as well to postpone any further discussion to the end of the next chapter when we have before us the relevant information concerning primary walls.

CHAPTER IX

The Primary Wall of Growing Cells

IT WILL be recalled that while we have defined the primary wall as the envelope surrounding the growing cell, this delicate membrane still remains present in an adult cell as the outer limiting layer of the wall. It is the purpose of the remainder of this book to attempt a description of the structure of the membrane while the cell is still growing, and to try to assess the present bearing of such a description on the processes of growth. We exclude from the membrane the middle lamella which cements two neighbouring cells together and all the layers which are subsequently deposited after growth has ceased. Such a separation between a growing and a non-growing wall is obviously one of considerable importance, associated with a variety of fundamental differences some of which are immediately obvious. Thus, since the primary wall is growing in area but not appreciably in thickness, while the secondary wall is growing in thickness but not at all in area, there must be some quite distinct difference between the two in relation to the protoplasm and, indeed, possibly to the metabolism generally of the cell. It has been said that cells change from a predominantly protein metabolism to a predominantly carbohydrate metabolism just at about the commencement of secondary wall formation. Though this bald statement can hardly stand nowadays without serious modification, for we know that carbohydrates must be manufactured rapidly in growing cells (see Fig. 3), nevertheless the distinction does hold even if not in the extreme sense first visualized. Concomitant with such a differential relationship, it will be a commonplace to those familiar with botanical material that the staining reactions of growing and adult cell walls can be quite different. For these, and for many other reasons, it is imperative always to treat the primary and the secondary walls as two different entities, and this view can be no more forcibly expressed than in these studies of structural relationships. It will become progressively clearer that at each and every step of a structural investigation the behaviour of the primary wall is radically different from that of the secondary.

This is not to say, of course, that the organization of the primary wall involves the embodiment of any new chemical species or the expression

of any new structural principle. On the contrary, the species of molecule built into the wall are precisely those found in secondary walls, with one outstanding omission—lignin. It is largely the relative proportion only of these substances which varies; and the structure of the component molecules remains as far as one can tell the same, though the relative disposition and the degree and type of orientation varies. This is therefore no new realm into which we are venturing. It is rather the same realm in a somewhat different guise and fraught with even greater difficulties.

The major obstacle to be overcome at the outset is to prepare the tissues in a state in which they can be studied by methods described here, without serious modification from the normal fresh condition. The material actually observed is preferably, or even more frequently inevitably, air-dried (for observation in the X-ray spectrometer) or dried in alcohol (for observation under the polarizing microscope). This latter is never absolutely essential, but since the precise interpretation of observations made with a polarizing microscope often depends on parallel observations made by the X-ray method, it is clear that most of the weight of interpretation falls on dried tissue. With secondary wall material this is no serious matter; one would not expect such rigid and endurable bodies to undergo much extensive change upon drying, and it has in fact been shown that such changes as desiccation produces are relatively minor. With primary walls, on the other hand, it is conceivable that drying will produce the most serious modification, and until such effects are very fully appreciated it is obviously dangerous in the extreme to be positive about any but the most general statements of structure.

With a mere chemical analysis, on the other hand, we are upon somewhat safer ground. The act of killing the cell by drying must, of course, change profoundly the inter-relationships of the various types of molecule present. Their amount and relative proportion, however, and to some extent their relative disposition within the wall, can hardly be materially affected. This is a point we might well take up before proceeding to a discussion of structure.

The chemical nature of the primary wall

It now seems without doubt that the bulk at least of all plants whose secondary walls contain cellulose also develop this polysaccharide in the growing cell, and that again this substance forms the framework around which the other substances are laid. Nevertheless there remain some peculiarities in this cellulose matrix. Some of these are structural

and will properly be discussed later, but there are some chemical aspects also which should be dealt with now.

Cellulose

Cellulose is usually recognized among botanists in virtue of the blue stain developed when the wall is treated with iodine after a preliminary swelling with sulphuric acid and, although there are cases in which both positive and negative reactions can be misleading, it nevertheless remains true that, with suitable safeguards, any variation in the staining can be interpreted in terms of the presence, modification, or absence of cellulose. Now the walls of many growing cells are found to stain in aqueous iodine alone. This was first observed by Ziegenspeck, who deduced therefrom the presence of a substance other than cellulose, which he called *amyloid* on account of the resemblance to the familiar starch reaction. Such walls, however, invariably give the normal cellulose stain as they grow older, and Hopman has observed intermediate conditions. Nevertheless this absence of a typical staining reaction in young cells, coupled with the difficulty of demonstrating the presence of cellulose by X-rays (see p. 174) led some workers to the suspicion that many, if not all, walls of very young cells were non-cellulosic. We shall see later that such an attitude is no longer tenable in the light of more recent X-ray investigations. It seems now highly probable that cellulose is present from the very beginning, but that the features by which it is normally recognized are masked by the particular conditions in which it grows. In general, it is necessary only to remove some non-cellulosic substance (often of a wax-like nature) in order for the staining reaction to change from negative to positive, and this differs from the condition in many secondary walls only in the smaller response of the smaller amount of cellulose. It seems now, for instance, rather certain that the *amyloid* of Ziegenspeck corresponds to a complex of cellulose chains only slightly different (*e.g.* shorter, more dispersed or in some different relation to incrusting substances) from that found in secondary walls.

The content of cellulose in primary is frequently much lower than it is in secondary walls (Table I) when expressed as a weight percentage of the total dry weight. Normally the primary wall is often swollen with water to a degree much greater than that reached by any secondary wall, with the exception of collenchyma cells, and this therefore implies a still lower volume percentage of cellulose in the fresh growing walls. It can be calculated that the volume percentage of cellulose in the parenchyma cells of oat coleoptiles (where vacuolation has commenced

so that the cells are nearing the end of their growth period) is of the order of 14% while in cambial cells (which are not vacuolated to any marked extent) it reaches the low value of only 8%. It is therefore clear why such walls are much less resistant to mechanical disturbance than are the adult cells, and it is immediately possible to attribute the ready dimensional changes undergone by growing cells in part at least to this low proportion of the structural polysaccharide.

Pectin, hemicelluloses and cellulosans

It has been known since the pioneer work of Mangin, beginning in 1888, that pectic substances are of wide occurrence in primary walls where they occur in high proportion. Normally they are completely insoluble in water and were originally termed "protopectin" or "pectose" to express this insolubility. The idea is now rather widely held that pectic substances in these walls occur largely in the form of the calcium-magnesium salts of partially methylated pectic acid, rendered insoluble either by the possession of long chain length or, and more probably, by entanglement, either mechanical or even chemical, with other molecular chains such as those of the cellulose. It has frequently been suggested that the preponderance of pectic compounds in growing cells may be connected with the high oxidation rate which is a feature of growth, the idea being that the enhanced oxidation may be connected with the development of —COOH groups in place of $—CH_2OH$. This would not, of course, necessarily imply that glucose, which would otherwise have gone to cellulose, is being side-tracked through an oxidation to pectin; the process must be much more complicated than that. The hemicelluloses, including the cellulosans, are commonly even more abundant in primary walls (Table I) and often take the form of xylan. Remembering the relation between cellulose and xylan demonstrated in the much more robust secondary wall, it may well appear that the peculiar reaction of the cellulose here may in some measure be due to heavy contamination with this very similar molecular species. Lignin is normally absent from primary walls, or present in such small amounts as to suggest that it enters the analysis as a contamination from the debris of neighbouring mature tissues.

Protein

Perhaps the most striking feature which comes out of any analysis of primary wall material is the frequent presence of protein. It has naturally to be remembered that the wall is in close association with the protoplasm within it, and that this involves some difficulty in separation. On this account some authorities have attributed the protein to a

contamination from the cytoplasm and have consequently denied its presence in the wall proper. Thus to quote only two cases, Wood (see references in 54) has denied that proteins may be present in walls to extents more than 0·001%, and Thimann and Bonner(54), while giving a figure of 12% in oat coleoptiles, allow that some of the protein may be present as a contaminant adsorbed from the protoplasm. Chemical analyses are therefore, by their very nature, suspect.

Staining reactions are usually more convincing though here again there are sceptics. Many observers, ranging from Krabbe in 1894 through Tupper-Carey and Priestley in 1923 to others up to the latest times, have claimed a positive staining reaction for proteins in young cell walls and there have been few dissentient voices in the present century. We shall see later that there are other, and quite undeniable, grounds for suspecting that proteins are present and that they may have a very pronounced effect indeed on the features involved in the increase of wall area associated with the growth process.

Other substances

Among the other substances which have from time to time been reported as present in primary walls, and of importance to their identification or their growth or both, we may perhaps notice the frequent references to substances of a wax-like character. A typical example of the effect of such substances is furnished by cotton hairs. Young cotton hairs (less than five days old) yield an X-ray diagram in which the characteristic arcs of cellulose are not recorded with an intensity sufficiently above the background level to be observed. The rather diffuse diagram which is given by these hairs disappears, however, if the hairs are treated with a wax solvent, and the characteristic diagram of cellulose then appears. It seems therefore reasonable to conclude that in the untreated hairs the cellulose diagram was masked by the overpowering wax diagram, but whether or not other complications are involved it is difficult to say.

On rather similar lines, the presence of phosphatides in primary walls has been claimed by Hansteen Cranner and occasional other workers and, though it has not perhaps yet been substantiated on a sufficiently wide range of materials and conditions, the presence of such substances does seem probable. It is known that the outer layers of the protoplasm do contain these substances and, now that we realize the close association between these outer layers and the wall itself, there seems every reason to doubt whether any such substance can occur in the one place and not in the other.

The general chemical picture of the primary wall is therefore one of considerable complexity. We certainly have present the polysaccharide cellulose, together with the other sugar derivatives normally found in association with it. In addition to these, however, there is almost certainly present a percentage, even if small, of protein and probably also of phosphatides. This leaves us with the vague suspicion that the primary wall may not after all be a passive coat around the protoplasm such as we have come to regard the secondary wall. We shall find, in fact, as we proceed to the investigation of the structural features in growing walls, that we have progressively more and more reason to suppose that the wall is, on the contrary, taking an active part in the increase in its own dimensions. Here, in fact, in the chemical analysis we have the first suggestion that the wall and the protoplasm at this stage constitute, if not one indissoluble whole, at least two interpenetrating complexes.

The X-ray diagram of primary walls

The earlier attempts to elucidate the structure of growing cell walls naturally made use of the less exigent conditions associated with observations under the polarizing microscope. In view, however, of the uncertainty in the interpretation of unsupported observations of this kind, and particularly since, with about only one exception, whole cells or even whole tissues were observed instead of the necessary single walls (see p. 116), it is preferable here to begin our analysis with the later, more rigorous interpretation of the X-ray diagram. Rather detailed studies have now been made both of the cambium of conifer trees and of growing oat coleoptiles, but since the results of the two investigations tally in all material points, only the former will be described here.

It is clear from what has been said that the first point to ascertain is whether or not the X-ray diagram which can readily be obtained under suitable conditions from dried tissue has any bearing upon the structure present in the fresh tissue. This is all the more essential in view of the recent complete denial of such a connection in cotton hairs.

Crystallinity in primary walls

Growth responses such as those elicited by small quantities of growth substances have frequently led to the speculation that the cellulose in the primary wall may not be associated into crystalline lattices as it is in secondary wall, and this seemed at first sight to have received strong support from the work of Berkeley and Kerr(55) on cotton hairs. They found that young cotton hairs photographed fresh from the boll, without any intermediate drying, gave no indication at all of the expected

cellulose diagram but, instead, only two vague rings characteristic of water in bulk. On drying, the water haloes naturally disappeared and at the same time the familiar cellulose diagram was manifested. If, instead of drying, the material was merely stretched while wet, then again the cellulose diagram appeared though not now so clearly on account of the presence of water haloes. Further, they showed that if the dried bundle of cotton hairs is now thoroughly re-wetted, then the cellulose diagram still remains clearly present. From this they concluded that the cellulose organization in the original fresh tissue was not such as to give an X-ray diagram, *i.e.* was not an association into the crystalline regions we have called micelles. In fact they went further than this. Since adult cotton hairs behave in precisely the same way, they denied the presence of these crystalline regions even in the fresh secondary walls.

Clearly, however, such an interpretation of these various diagrams cannot in the least be regarded as final. In the first place, the absence of an X-ray diagram does not at all imply that there are *no* crystalline regions but merely that those which do occur are too small in width (estimated to be somewhat less than 20 Å.) to give a crystalline pattern. More seriously than this, however, the authors evidently paid too little attention to the possibility that the cellulose diagram in fresh material was being masked by the water haloes. This might readily explain the whole series of observations, particularly since in the fresh material the lumina of the hairs would be full of water, whereas in the dried, re-wetted, sample they would probably be collapsed. Observation of other material has, in fact, led to a conclusion quite opposite from that of Berkeley and Kerr.

As regards the condition obtaining in the thicker secondary wall, it was clearly desirable to repeat these observations on cells with walls so thick and so well crystalline in the dried condition that the water haloes had little chance of masking any diagram in fresh material. Perusal of this book will show that the alga *Rhizoclonium* was admirably suited to this purpose. Using this material, it was found that samples taken straight from the pond, photographed without drying and kept wet with running water during the whole of the exposure, yielded precisely the same diagram as did the same sample after drying (56). This disposes at any rate of the generalization of the interpretation of Berkeley and Kerr. With primary walls, similar observations proved impracticable, presumably on account of the high water-content of growing tissues in terms of the amount of cellulose present. Nevertheless with conifer cambium it has been shown that, while fresh cambium photographed wet gives only water haloes, the same tissue photographed in

an atmosphere of relative humidity 98% gives also diffraction rings typical of cellulose, presumably unmasked by the reduction of the intensity of the water rings, in exactly the same disposition as later found from the dried tissue(56). It is not to be denied that the removal of free water consequent upon equilibration with an atmosphere of 98% relative humidity may have caused some modification of structure; but it is strongly to be questioned whether this comparatively small degree of drying could materially affect the crystallinity of the cellulose. This correspondence of the photographs of wet and dry tissue will therefore be taken here to imply that the broader details at least of structure in the walls of fresh tissue can be deduced from observation of dried material.

Orientation of cellulose in primary walls

When the cambium is stripped from a tree as described previously (p. 154) then the resultant ribbons of tissue are far too thin to yield an

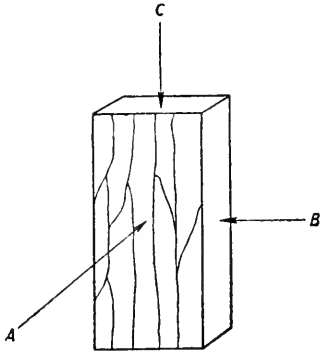


FIG. 59. Diagrammatic representation of a composite block of flattened strips of cambial tissue.

Direction *A* lies normal to the flat faces of the strips (*i.e.* along a direction radial to the trunk when the strips were in position in the tree).
Direction *B* lies parallel to cell length, and direction *C* is mutually perpendicular to *A* and *B*.

X-ray diagram. If, however, several such strips are dried on to glass and subsequently piled on one another in such a way as to maintain the parallel orientation of the cells from layer to layer and the resultant bundle is irradiated in the direction *A* (Fig. 59), then this bundle yields a diagram such as illustrated in Plate IX, Fig. 2. The implications of such a diagram will be immediately obvious. On drying, the cells collapse and become flattened in the plane of the strips (parallel to the glass surface) so that in effect the block corresponds to a series of

parallel walls. The very obviously greater intensity of the arcs visible along the meridian therefore, indicates immediately that the molecular chains of cellulose are oriented more or less transversely in the cells. The continuation of these arcs around a complete circle shows further that this orientation is far from perfect: that while the general trend is for the chains to lie transversely, there is a good deal of spread about this transverse position. Figure 60 will give some idea of the type of structure which may be deduced from such observations. It will further be noticed that if the chains had been truly transverse then the X-ray diagram resulting when the beam is directed in turn along *B* and *C* (Fig. 59) should have differed; along *C* a ring diagram should have been obtained since the beam would then point along the chains, whereas position *B* should have yielded a good fibre diagram. Since, in fact, these two diagrams are identical (Plate IX, Fig. 3) then this implies that there must be some considerable dispersion about a nearly transverse general direction. From these two diagrams it has been calculated that the average angle between the cellulose chain direction and the transverse plane in the cells cannot be greater than 16°(57). We may note in passing that, even if the cells had not completely flattened on drying, comparison of Plate IX, Fig. 2, with the diagrams of the model spirals (Plate VI, Fig. 7) show quite unequivocally that the cellulose chains must still be directed in a very slow spiral.

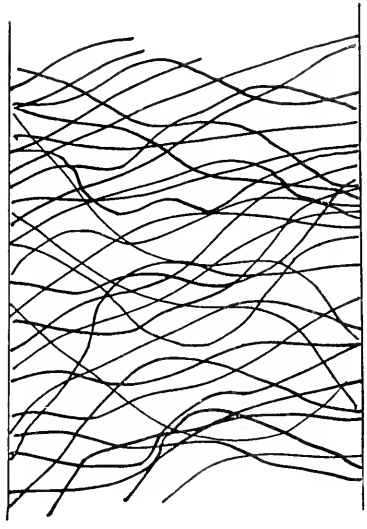


FIG. 60. Diagrammatic representation of the run of fibrils (full line) in a primary wall. The length of the cell is supposed to lie parallel to the edge of the page.

Observations under the polarizing microscope lead to precisely the same conclusion. In face view, the m.e.p. of single walls lies almost transversely (the exact direction being difficult to ascertain in view of the low birefringence) and the wall is nevertheless birefringent even when viewed in optical longitudinal section, *i.e.* when the side walls of a whole cell are examined. This must mean that the cellulose chains lie almost transversely with probably considerable angular dispersion. Again, it

has been found that, in thin transverse sections, the birefringence of the walls amounts to about $0{\cdot}001_6$. Now, taking the percentage of cellulose as 25% (Table I) and ignoring small density corrections, we may say that

$$n_\gamma' = 0{\cdot}25n_\gamma + 0{\cdot}75n_r,$$

$$n_\alpha' = 0{\cdot}25n_\alpha + 0{\cdot}75n_r$$

and

$$n_\gamma' - n_\alpha' = 0{\cdot}25\,(n_\gamma - n_\alpha),$$

where n_γ' and n_α' are the major and minor refractive indices of the wall in transverse section, n_r is the refractive index of the (isotropic) contaminant and n_γ and n_α are the refractive indices of the cellulose micelles as seen in transverse section of the wall. Taking the angle of the chains to the transverse at the maximum value of 16° (and therefore calculating the minimum possible birefringence) then $n_\gamma - n_\alpha$ should be about 0·047 assuming the micelles to lie all parallel, and hence

$$n_\gamma' - n_\alpha' = 0{\cdot}012.$$

Since the observed figure is so much lower than this then there must be either high angular dispersion or a very low crystalline to non-crystalline ratio.

While we are considering the orientation in the cambium let us note in passing that the primary wall is still present around the mature tracheid, where with care it can be stripped off and examined. This has been done (58) and it is found that, as judged both by the m.e.p. and striation direction, the cellulose chains make on the average an angle of about 10° to the transverse both in *Pinus radiata* and *Pinus longifolia*, the only two cases in which a direct measurement has been made. Van Iterson had, indeed, said somewhat earlier that the m.e.p. of such walls were almost transverse in a variety of conifers.

Returning to the X-ray diagrams, however, there are some other features of considerable further interest. It will be noticed that the arcs are much more diffuse radially than are those presented hitherto in cellulosic specimens. Such broadening of arcs implies less perfect crystallinity, but it is not easy at the moment to define the type of imperfection involved. On the original Micellar Hypothesis, it is possible to say that the micelles here are much smaller than in the secondary walls, and even in the modified hypothesis the same interpretation could be advanced. In these terms, it can be calculated that the micelle width is of the order 20–30 Å. and so lies towards the lower limit of the size of crystallite which can give crystalline X-ray diagrams.

It is equally possible that the broadening is due, of course, to random imperfections in the alignment and spacing of the cellulose chains without the necessity for the assumption of smaller specific crystalline regions.

Taking the value of 20 to 30 Å. at its face value, however, one can make some interesting calculations as to the disposition of the micelles. Thus, if these are taken to be d cm. in diameter and of indefinite length, spaced uniformly by a distance m cm., then in a 1-cm. cube there will be a total micelle volume of $\pi d^2/4m^2$. This is the relative volume of the cellulose, which is known to be about 8%. Hence

$$d^2/4m^2=0{\cdot}08,$$

and therefore

$$m=3{\cdot}3d.$$

The intermicellar distance would then be 70–100 Å. or, if only 60% of the cellulose is crystalline, the distance would be of the order 90–120 Å. The actual distances would be very variable, of course, but this little calculation is very instructive in showing how widely spread the cellulose matrix must be in these growing walls. It is also interesting to note that, if the wall could be considered as consisting of individual cellulose chains arranged strictly parallel to, and equidistant from, each other, then the distance between them would be of the order of 13 Å. This is of the order of three molecular diameters and compares favourably with the bimolecular water layer separating the chains as suggested by Berkeley and Kerr.

We have then here, good evidence that the cellulose chains in primary walls do show some orientation and that, on the whole, the orientation tends to be in a flat spiral. The same conclusion can also be drawn from the growing walls of other cell types. Thus in the sporangiophore of *Phycomyces* the chitin chains in the (apical) growth zone are oriented in a spiral whose angle to the transverse is around 143° (62) (see p. 189) and in cotton a somewhat similar orientation has been equally clearly demonstrated (51). Orientation of this general kind has also been claimed for the parenchyma of *Helianthus* hypocotyls, staminal hairs of *Tradescantia*, sclerenchyma cells and collenchyma cells, and for the arms of stellate pith cells (see references in 58). There are occasional exceptions—for instance the primary walls of jute and hemp fibres at a late stage of growth and the outer walls of epidermal cells in oat coleoptiles —but the imposing array of growing cells with the same type of transverse orientation has led to a good deal of speculation as to the connection between orientation and growth processes. Two problems face

us immediately (*a*) what causal factors underlie this ubiquitous transverse orientation, (*b*) what happens to the orientation during growth? We have already a partial answer to this last question since in the work described above no attempt was made to choose any particular stage in growth, so that it appears that the orientation is invariate. It is nevertheless worth while to take up each point in turn since a good deal of interest will emerge from the discussion. This discussion will, however, take us somewhat beyond the bounds of established knowledge and is therefore best made in a separate chapter.

CHAPTER X

The Mechanisms of Orientation and Growth

As a result of observations such as those mentioned above it is frequently stated in the literature that in growing cells the molecular chains of cellulose align themselves almost perpendicularly to the direction of highest growth rate. This generalization is clearly pardonable since the bulk of the cells investigated have in fact been elongating. In one instance, however, the cells of the cambium, the cells increase in radial diameter much more rapidly than they do in length (though this increase is masked by repeated divisions). Unless such a case can be regarded as a complete exception—and such a view is most repulsive—then it follows that a cell can increase equally well either longitudinally or transversely and that the form of the cell might therefore be after all controlled by some factor other than the molecular orientation within the wall.

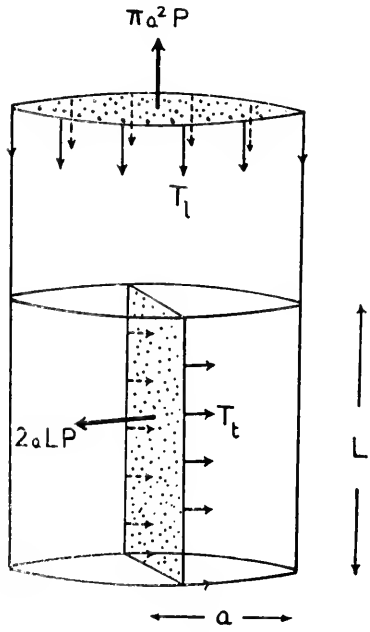


FIG. 61. For explanation, see text.

It is interesting that the more recent attempts to explain orientation in growing walls have centred around the effects of the shape of cells on the tensions in their walls induced by internal hydrostatic pressures. The earlier suggestion of Denham that protoplasmic streaming was involved in the alignment of wall particles (much as the chains in cellulose solutions are aligned in the manufacture of artificial silk by extruding through fine jets) was adequately disposed of by Martens, and the later modification by van Iterson, though ingenious, proved no more successful (see references in 4 (*b*) and 57). There is today no serious consideration

being given to such a hypothesis. On the other hand a second type of suggestion has been made more recently both by Castle in America and van Iterson in Holland.

The suggestion starts from the known distribution of stress in the walls of a hollow cylinder submitted to uniform pressure from within. We can perhaps take up the ideas most easily by considering the case of a gas cylinder containing gas under pressure. It is well known that if such a cylinder bursts on dropping, then the wall does not usually splinter nor is one end blown off; instead, the cylinder splits down the middle like a pea-pod. Evidently, since the tensile properties of the metal wall are the same in every direction, this implies that the transverse stress in the wall is greater than the longitudinal stress. It is easy to calculate that the stress is indeed twice as great transversely as longitudinally.

Thus consider a cylinder containing fluid under pressure P, and consider first the equilibrium conditions of a septum inserted transversely (horizontal area shaded in Fig. 61); the upward force acting on the septum is $\pi a^2 P$. The balancing force directed downwards is derived from the tension in the wall, and this amounts to $2\pi a T_l$, where T_l is the longitudinal tension per unit of the wall periphery. Hence

$$\pi a^2 P = 2\pi a T_l,$$

so that

$$T_l = \frac{P}{2a}.$$

Considering now a longitudinal septum (the longitudinal shaded area in Fig. 61), the two balancing forces are now $2aLP$ and $2LT_t$ where T_t is the transverse tension per unit wall length. Hence,

$$2aLP = 2LT_t$$

so that

$$T_t = \frac{P}{a}.$$

Thus the transverse tension is twice the longitudinal tension. Now the suggestion is that, since in a cylindrical growing cell the tension transversely is twice as great as longitudinally, it is for this reason that the cellulose chains come to be oriented transversely.

Unfortunately such a mechanical explanation will hardly bear examination and a good deal of criticism has been forthcoming. These criticisms may be classed under three headings: (*a*) the difficulty of connecting *stress* alone with orientation and the inadequacy of the

observational evidence to associate the two together; (*b*) the difficulties encountered when attempting to interpret orientation in non-cylindrical cells; and (*c*) the inadequacy of the hypothesis when secondary walls are examined.

As regards the first, the published support for the hypothesis invariably tries to associate orientation, not with stress but with the resulting strain. Thus, if a series of squares are drawn on a partly inflated cylindrical rubber balloon, then they become rectangles with their longer sides transverse when the balloon is further inflated. This, however, is merely because in such a balloon expansion is taking place more rapidly in the transverse direction than in the longitudinal on account of the anisotropy of stress—and this is not the condition obtaining in elongating cells, for the cells always increase their length/breadth ratio. Again, to take one example of the evidence put forward by observation of growing cells, let us consider the evidence brought forward by Maas Geesteranus. The pith cells of *Juncus* develop hollow cylindrical protrusions which elongate as the cell grows. These are therefore quite separated from each other and from other cells and can be studied individually in sections. Maas Geesteranus has measured the phase difference (p. 69) of the (double, upper and lower) walls of these cells and has found a relation between this phase difference and the length of the arms as shown in Fig. 62. The increase in phase difference (which is such that the m.e.p. is directed transversely) he attributes to an increasing preference of the cellulose chains for the transverse orientation (for which there is in any case no evidence since increase in wall thickness, cellulose content, etc., would lead to the same result) and this in turn to an increasing strain during growth. The observed correlation, however, is with *length* and there is no evidence in the drawings published by Maas Geesteranus that any appreciable transverse strain occurs at all. If strain had any appreciable part to play in orientation, one would have expected the chains to become more nearly longitudinal during growth. Indeed, it would seem much more reasonable to conclude that the cell remained cylindrical *because* the chains remain transverse than to attribute transverse orientation to the cylindrical shape. Even this can be doubted, as will appear later.

Under the second heading we may note that in *Cladophora* we have transverse orientation *par excellence* although the growing tip of a filament is dome-shaped and therefore not subject to extreme anisotropy of stress and that, lower down in the tip cell, and in cells lower in the filament, lamellae with transverse orientation are repeatedly sandwiched between others with almost longitudinal orientation although the cell

remains cylindrical. Again, the alga *Valonia* never was other than approximately spherical in shape and nevertheless it lays down cellulose, with great facility, transversely oriented to the (somewhat vague) axis of symmetry.

Finally, under the third heading, we may note that in the elongated cells discussed in Chapters VII and VIII, the secondary wall was deposited also during the continued presence of hydrostatic pressure in the cell; and nevertheless the orientation is far from transverse.

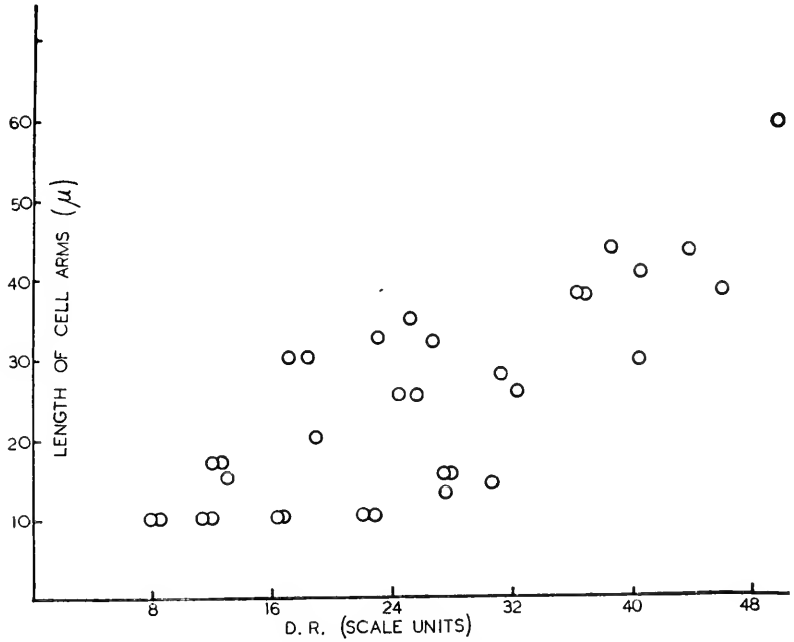


FIG. 62. For explanation, see text (after Maas Geesteranus).

Attractive though it is to attempt to correlate growth form with known physical principles, this particular aspect must at the moment be abandoned, and the explanation of transverse orientation in growing walls —and indeed of orientation in walls generally—must be sought along other lines. Discussion of these may well be postponed until the impact of growth on wall organization has been explored.

The invariate orientation during growth

The above discussion already makes it rather clear, and it has already been mentioned briefly, that growth has remarkably little effect on orientation. This point has been investigated specifically by several

workers and the work of Bonner(77) may be taken as an example. Bonner observed the phase difference of half coleoptiles, obtained by splitting the coleoptile in two longitudinally and removing the epidermis. Since the sign of the phase difference was such that the m.e.p. must lie transversely, he concluded that the cellulose chains in individual walls must also lie in this direction. Reference back to Fig. 31 will show that this conclusion would not be justified even if single cells had been examined (since both front and back walls are involved), and at the time the author cast doubt on the validity of this interpretation by showing that, if individual walls are examined, then the m.e.p. lies in a slow spiral. Now, however, we know from X-ray analysis that the angle of the cellulose chains to the transverse in coleoptiles between 2 and 3 cm. long is not greater than 16°, the transverse orientation can be accepted as an approximation. The coleoptile grows from a length of 1 cm. to a length of about 3 cm. in light (or about 6 cm. in darkness, see Fig. 3, p. 15), solely by the increase in length of the individual cells due to absorption of water. Bonner showed that during this process the phase difference of half coleoptiles remained constant with the m.e.p. transverse, even after the cells had extended some 100%. He interpreted this as implying that the constituent cellulose chains also remained transversely oriented. Further investigation showed that if the halved coleoptiles were stretched mechanically by only 9%, then the m.e.p. became longitudinal and he took it that a reorientation had occurred in the direction of stretch. He therefore concluded that no reorientation occurred during growth and that growth of a wall is something different from passive extension under mechanical forces.

This orientation story, however, leaves much to be desired. The maintenance of transverse m.e.p. during growth might well have meant only that the angle between the chains and the transverse never exceeds 45°; and one can hardly imagine that mechanical extension of only 9% could possibly change the orientation from almost transverse to almost longitudinal. It seems much more likely that this latter effect is to be ascribed to photoelastic phenomena, resembling the birefringence induced in glass by straining, but nevertheless his general conclusion still stands. If stretched walls show photoelastic effects while growing walls do not, then growth can hardly be regarded as passive elongation under tension. These observations, and several others like them, do therefore indicate most clearly that the processes of growth must be such as to throw little strain on the wall and therefore to lead to little reorientation of the cellulose from the transverse direction.

Further evidence can now be adduced from the studies of the algae

mentioned in Chapter VI. In *Hydrodictyon*, the smallest cell examined possesses a wall in which the cellulose chains are oriented at random, and no matter how long the cells become the orientation remains random, so that this elongation is presumably associated with some factor other than tension due to turgor forces. In another sense, the existence of the two algae *Cladophora* and *Spongomorpha* with the same growth form but vastly different wall structure, points in the same direction. Nevertheless some caution is to be exercised, for there are isolated cases in which growth phenomena do seem to involve tensile effects in the walls.

No more striking example of these latter cases can be taken than the growth of the sporangiophores of *Phycomyces* as first investigated by Oort and Roelofsen and later by Castle(59). Thanks largely to the meticulous work of this latter investigator, a great deal is now known of this matter and only the gist of it can be given here.

Spiral growth in the sporangiophores of *Phycomyces*

The sporangiophore grows towards the light from the substrate as a thin cylinder, whose wall is composed of chitin impregnated with protein and other substances. The peculiarity which has led to so much effort being put into its investigation is that during its growth period it not only elongates but also twists around its own axis. This twisting, however, is not noticeable unless markers, in the form of light glass fibres or *Lycopodium* spores, are placed upon the tip of the sporangiophore and observed over a period of time. Growth occurs only in the apical 2 mm. or so of the sporangiophore, where the wall remains thin and may be called a primary wall. Below this growth zone, secondary wall deposition effectively prevents any further elongation. The progressive elongation of the growth zone, and the continual removal from it of its lower section by deposition of these secondary layers, maintains the depth of the zone more or less constant. There is no morphological twist such as may be seen in, for instance, bindweed twining round a support: the twist is achieved through displacements of the wall substance at the molecular level.

At this period, before the sporangium has begun to appear, the cell twists left handed, *i.e.* looking down on the tip of the sporangiophore, a marker will move in a clockwise direction. The onset of swelling in the sporangium calls a halt both to elongation and twisting and this marks the end of what is called Stage I. Once the sporangium is fully swollen (Stage II), the whole structure rests for a time (Stage III) and then once again begins to elongate and to rotate. Now, however, the

twist is right handed and continues to be so for anything up to two and a half hours, varying with the particular sporangiophore observed. During this time the rate of twisting is slowly diminishing; it finally comes momentarily to a halt and then left-handed twisting again develops, reaching a constant rate which thereafter continues for several hours.

Now the sporangiophore is simply a hollow tube of chitin filled with liquid, or semi-liquid, cytoplasm containing a vacuole, and we can hardly escape the conclusion that these peculiar effects of growth must be due to some feature of the wall. This view was indeed expressed very early in the study, both by Castle himself and by Heyn. We may think of the organization of a chitinous wall as resembling very closely that of the cellulosic walls we have been considering up to now, with molecular chains arranged parallel to each other in "micelles", and so on, except that the unit of structure is not now β-glucose, but a derivative, acetyl glucosamine (Fig. 63).

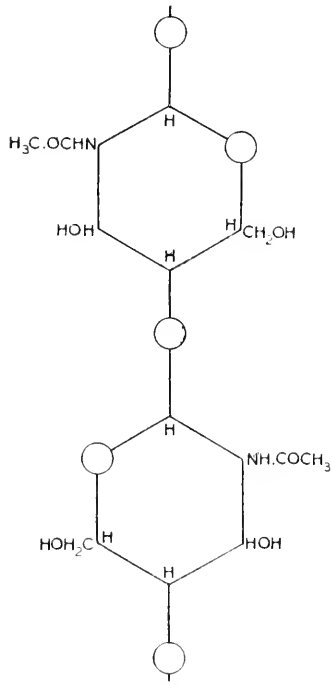


FIG. 63. Two glucosamine residues in a molecular chain of chitin.

Castle suggested that the twisting might be due to the anisotropic response of such a wall to the hydrostatic pressure within the cell resulting from its turgor. This is undoubtedly along the right lines, but naturally cannot lead to any quantitative check until much more consideration is given to the various factors which may be involved. The suggestion of Heyn that twisting could be due to failure of the wall under stress, along slip planes predetermined by the crystalline structure of the chitin, can hardly be accepted since in that case the rate of twisting should be rigidly constant—which is by no means what is observed.

In attempting to derive an interpretation which may be satisfactory in a quantitative as well as a qualitative sense, it is obviously necessary to determine accurately the rate of rotation and of elongation under a variety of conditions. This has been done in a series of classic papers by Castle. Next, it is imperative to define the orientation of the chitin

chains in the growth zone and to make at least some estimate of the elastic properties of the wall in that region. Both have now been achieved.

From the time of the first publication in 1931 by Oort and Roelofsen, it was known that the chitin chains in the growth zone are oriented almost transversely and, although this has since been questioned from time to time, there can now be no doubt of its general validity. The earlier observations were made solely under the polarization microscope and such observations with chitin are difficult to interpret unambiguously both on account of the low birefringence of the walls and of the sensitivity of the sign of the birefringence to the nature of the medium in which the material is immersed for observation. The case clearly called for analysis by X-ray methods. This, however, involves the careful drying of many sporangiophores which must then be lifted from the glass on which they are attached and piled one on another, keeping the growth zones coincident and in careful parallel alignment, until a bundle of least 0·5 mm. thick is obtained. Unfortunately these sporangiophores tend to distort badly if dried without some considerable care, and, when dried, are extremely fragile. The author is therefore fortunate indeed that the delicate manipulative skill of Mrs. Middlebrook, working in his laboratory, has built up a suitable bundle, the X-ray diagram of which shows that the chitin chains do run almost transversely. Following this, observation under the polarizing microscope by the methods described on p. 61 *et seq.* (with the use of an arc lamp in place of the normal microscope lamp on account of the low birefringence) has shown that in Stage IV sporangiophores the chains make an angle to the transverse of the order of 14°.* With this information we can proceed to consider an interpretation of spiral growth which was, in fact, put forward (60) before the structure of the wall was known with certainty. It is not possible here to go into any detail so that only a brief summary will be given. Further information can be obtained from the original papers (60, 62).

The wall in the growth zone consists, in effect, of a number of flat spirals. Now we know that when a flat spiral spring is extended so that one end is free, then this end twists. In a spring of radius a in which the winding is circular in section and makes an angle α to the transverse (Fig. 64) then if the spring is loaded axially as shown the rotation of the end, $\Delta\phi$, for an elongation of ΔL is given by the relation,

$$\frac{\Delta\phi}{\Delta L} = \frac{\cos\alpha \sin\alpha\,(1-2n/q)}{a[\cos^2\alpha+(2n/q)\sin^2\alpha]}, \qquad ..(1)$$

* Considerable care has to be exercised here, since chitin possesses *negative* intrinsic birefringence coupled with (usually overriding) *positive* form birefringence.

where q is the Youngs modulus of the winding and n the torsional rigidity. Strictly speaking, this relation holds only in cases where the winding is isotropic, but since we are considering here the value of q for extensions parallel to the winding only, and of n in a plane at right angles to this, then the errors involved are presumably quite small. There is naturally some hesitation in comparing such a static model with the dynamic state in the growing sporangiophore, but there are grounds for thinking that these two are not so dissimilar as might at first appear. We notice first the following qualitative points:

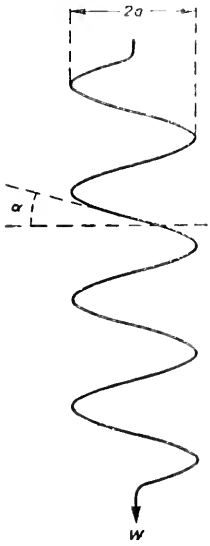


FIG. 64. For explanation, see text.

(1) The relation is satisfactory in that it throws the weight of the explanation on the wall, where it undoubtedly belongs. The sporangiophore is known to be under hydrostatic pressure from within, and the impact of this on the closed end corresponds to the axial weight in the model.

(2) Elongation and rotation go always together, rotation stopping when elongation ceases, and resuming immediately growth begins again.

(3) The rate of rotation can be very variable since it depends not only on the rate of elongation but also on the value of α, q and n. The latter two factors are known to vary widely in substances of this kind.

The relation is equally satisfactory in a semi-quantitative sense. Thus the following comparisons with observational data have been made:

(1) From observed rates of elongation per unit elongation ($\Delta\phi/\Delta L$) and the known value of α it has been calculated that $n/q=0{\cdot}22$. This lies well within the range of values recently found for the chitin of the mature sporangiophores (62), and agrees with the several estimates which have been given for cellulose. While no particular stress can at the moment be placed upon this correspondence until growing walls have been investigated, it is nevertheless satisfactory that the value of n/q required to explain spiral growth quantitatively is of the correct order of magnitude.

(2) Castle has observed the externally applied torque required just to stop rotation. He found that the torque varied rapidly with cell diameter. The present theory predicts that it should vary with a^3 and gives

a satisfactory quantitative check with Castle's actual figures. Furthermore the torque required can be calculated roughly, and the calculated figure again agrees with the observed.

(3) Finally we come to the most searching test—the explanation of the reversal of spiralling at the renewed onset of growth after the sporangium has developed. The sign of $\Delta\phi/\Delta L$ depends upon the sign of $(1-2n/q)$ and in left-hand spiralling is positive since $2n/q$ is less than unity. If, however, q sufficiently decreases at any time or if n increases, then $2n/q$ might become greater than unity, when $(1-2n/q)$ would be negative and the spiralling would therefore reverse. There is therefore here a possibility of explaining reversal, and the particular explanation which has been put forward, which is not the only possibility, is this. As new chitin particles are intercalated in the wall their mutual orientation will be improved by the increase in area of the wall. This will maintain both n and q at a certain level. Once elongation has ceased, however, further new deposits will no longer undergo this improved orientation. The value of q which is known to be very susceptible to orientation (Table IV), will decrease and of n probably increase so that when growth recommences the value of $(1-2n/q)$ may well be negative. The cell will then rotate in a right-hand spiral. Growth will, however, progressively increase the degree of parallel orientation, so that q will progressively increase and n diminish, until the final values are closely similar to those obtaining before elongation ceased. Hence the right-hand spiralling will slow down and finally revert to left hand.

No quantitative check of this latter effect has yet proved possible, and there are other possible explanations of the reversal within the framework of the theory, some of which have been pointed out by Roelofsen (see refs. in (62)). It is worth noting, however, that cessation of growth at any time should cause a reversal if the present theory holds, and it is therefore exceedingly satisfactory that it has recently been shown, again at the hands of Mrs. Middlebrook, that when growth is temporarily stopped in late Stage IV sporangiophores by immersion in very weak detergent solutions then, on the resumption of growth, the growth spiral is first right-handed and then slowly reverts to left-handed, just as it does in the normal phenomena associated with the early Stage IV condition (62) (Fig. 65).

Finally we should add that a further check has been made that the relation between $\Delta\phi/\Delta L$ and a, the diameter of the sporangiophore, is of the kind predictable from equation (1), p. 189 (62).

It is therefore possible in this particular case that some stages of growth do involve the tensile properties of the wall, and it seems likely

that the same is true of the majority of other growing cells. The electron-micrographs of the wall of *Phycomyces* recently published by Frey-Wyssling(73) reveals a structure in the primary wall a good deal more complex than had been supposed, though his conclusions are supported neither by the electron-micrographs of Roelofsen nor of our own (62). It is certain that the present proposals are based on far too simple premises, but nevertheless we may have some confidence that they are expressed in terms of the correct and relevant variables in the wall.

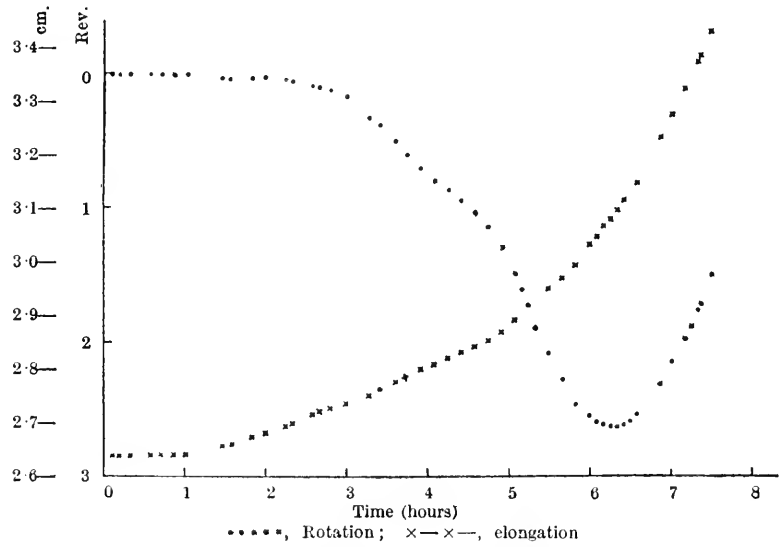


FIG. 65. The rotation and elongation of a sporangiophore of *Phycomyces* after a temporary cessation of growth caused by immersion in a 0·002% aqueous solution of dodecyl trimethylammonium bromide. Note that the rotation changes sign at the minimum on the rotation curve.

It is very clear, however, even with *Phycomyces* that this is not the whole story, and it seems possible that these reactions which can be explained in terms of wall extension are in a sense the aftermath of the more fundamental stages in growth. Thus, if a growing sporangiophore is immersed for a short time in a solution of a suitable dye (62) then the whole wall of the growth zone becomes deeply stained. If the cell is then allowed to grow, the growth zone becomes progressively less stained as the young wall present during staining is left behind, becoming part of the adult wall by deposition of secondary lamellae, and is replaced by new wall developed from above. When the sporangiophore has elongated by about 2 mm. (the length of the growth

zone) the growth zone is completely unstained. This type of observation implies that the production of new wall occurs only at the very tip of the sporangiophore, in Stage IV therefore immediately below the sporangium itself (62). Here, indeed, is the only locality in which the "transverse" chitin chains are not associated with longitudinal chains, as judged by the X-ray diagram (62). It is at the moment impossible to be more specific, but it does seem highly probable that the insertion of new wall material does not occur uniformly over the whole growing region.

Wall structure and cell shape

An examination of the inter-relationship of growth and structure from another point of view is even more striking. It has always been a puzzle why cells which are approximately isodiametric develop into long cylindrical forms. The advent of the new knowledge of structure derived by the physical methods described in the previous chapters soon pointed the way to a ready explanation of this phenomenon, an explanation which is no doubt widely held today. We can see now that this cannot be accepted except in a very general sort of way. With cells like those in coleoptiles it is dangerously easy to conclude that, since the chains of cellulose are oriented transversely, then the cell will naturally enlarge more readily longitudinally, since the resistance to elongation of cellulose is much less at right angles to the chains than parallel to them. Even here a warning note is sounded, for in the cambium, which has almost precisely the same structure, the bulk of the dimension change is transverse, leading to the frequent longitudinal divisions. It is very instructive to repeat an observation made some years ago by Tupper-Carey and Priestley. If the bark of a tree is removed as shown in Fig. 66 in such a way that the upper and lower parts are joined only by horizontal strips, then the behaviour of the cambium in these strips is most peculiar. The cambial cells, which are originally elongated in the vertical direction, undergo first repeated transverse divisions cutting each cell into a number of isodiametric cells. These then begin to extend in a horizontal tangential direction so that after a time the cambial cells are reoriented in a horizontal direction; they continue to produce tracheids of the wood, but these are at first isodiametric and then elongated horizontally. We have repeated and confirmed these observations on several occasions, and it is obviously very difficult indeed to harmonize such behaviour with the idea of cell form as a reaction to wall structure.

The story in the algae is even clearer. With *Hydrodictyon* it is difficult to see why the cell with such a wall fails to grow into a balloon.

Finally, the genus *Cladophora* presents an even more striking case. We have seen that in the large majority of species the wall structure resembles closely that of *Valonia*. Whereas, however, *Valonia* develops into a large bubble-like cell, the *Cladophoras* have adopted the filamentous habit. Even more striking, in *Spongomorpha*, closely related to *Cladophora*, the chains of cellulose are oriented at random. Here then we have two almost identical growth habits with entirely different wall structure. Incidentally, this difference in wall structure in two species

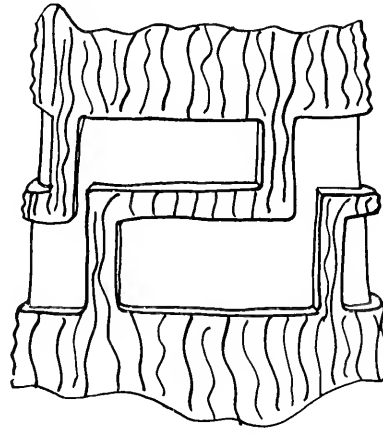


FIG. 66. For explanation, see text.

which were once allocated to the same genus raises problems of its own, but this is not the place in which these can be discussed.

Osmotic forces in growth

Nor are the anomalies in current ideas confined to the structural aspects of the growth process. If dimensional changes in cells were effected through the mechanical strains in the wall induced by the internal hydrostatic pressures, then we might expect the hydrostatic pressure to be greater in a cell when growing than it would be during resting periods. That this is not always so is at least indicated in the work of Burström (63(*a*)). His results show most clearly that when a cell begins to grow the internal pressure diminishes, and while we must await confirmation of these results before expressing a definite opinion, this observation tends to throw even more doubt on the conception of dimensional changes in cells as a reaction to mere mechanical influences. It would, of course, be natural to conclude that if the pressure does not increase, then the resistance of the wall might decrease. We

have now seen, however, that this still does not take us out of the morass.

Frey-Wyssling (63(*b*)) has recently made use of the plasmolytic data of Burström (63(*a*)) in growing wheat roots to demonstrate an increase in the extensibility of the wall during the phase of growth by vacuolation. Taking the length of a turgid cell as $(l+\Delta l)$ and of a plasmolysed cell as l and calculating the tension in the wall, T, due to the internal turgor pressure, he defines a value E such that

$$T=E\frac{\Delta l}{l}.$$

Formally E is then analogous to Young's Modulus and Frey-Wyssling does use this term. It seems, however, most improbable that the tension/elongation relationship during plasmolysis is linear as the above equation would suggest, and it is obviously undesirable to be precise as to the definition of E. Its value does decrease most markedly during the early growth phase; but rises again very steeply with no obvious diminution of the rate of growth. It is therefore impossible to discern in this elegant piece of work any clear correlation between growth rate and tensile properties. Indeed, though a final decision must await more precise data obtained in such a way as to be more readily interpreted in terms of growth, it seems at the moment difficult to avoid the conclusion that the tensile properties of the wall have very little indeed to do with the regulation of growth.

The cellulose-protein complex in growing walls

Clearly we need to know a good deal more about the wall during the growing phase, and some attention has been given to this problem. Let us return for a few moments to the X-ray investigation of cambial cells and oat coleoptiles briefly described above. In untreated cells it is difficult to make out any arcs on the X-ray diagram corresponding to the 5·4 and 6·1 Å. spacings so typical of the normal cellulose diagram, and even the arc corresponding to 3·9 Å. is very diffuse. If, however, pectin or protein or even water are removed from the wall then the diagram becomes much sharper, and an arc appears at 5·5 Å. which probably corresponds to a fused 5·4, 6·1 Å. reflection. This can mean only that the cellulose in the wall is associated closely with all these substances, and recalls in particular the chemical evidence, supported by staining reactions, that proteins are present in growing walls. From this it is an easy step to the conception of the wall at this stage, not as an enclosing sheath which reacts passively to stimuli from within the

cell, but as part of the whole growing organism: that the so-called wall, in fact, represents nothing more than the outer layers of the protoplasm within which cellulose is deposited. This is not the first time that this possibility has been mooted but it is the first time that it has been suggested on purely crystallographic grounds.

The elegant electron-micrographs recently obtained by Mühlethaler and Frey-Wyssling(41) can lead to precisely the same conclusion. In these photographs, threads appear which look precisely similar to those previously found in the author's laboratory in the *Valonia* wall, and, in particular, are again rather uniformly about 250 or 300 Å. wide. Whether these can be strictly identical, in view of the difference in sharpness of the arcs in the corresponding X-ray diagrams, is a point which has already been discussed (p. 90). The threads, however, are arranged in much more random fashion than are those in *Valonia*; but they are also in parts *intertwined.* It is not therefore possible to imagine them as being oriented on a flat protoplasm-wall interface, and the simplest hypothesis would be that they are spun out of a cellulose-cytoplasm complex. Somewhat the same intertwined condition has now been shown in *Valonia*, so that the idea of orientation at an interface may have to be discarded altogether (42(*d*)).

The mechanism of orientation and the growth process

This structural complexity in a growing wall suggests at once that the two problems discussed in this chapter—that of the mechanism of orientation and that of the maintenance of this orientation during growth—are but two aspects of one and the same problem and that therefore if the one is solved, so is the other simultaneously. Just as, therefore, we are unable to accept purely mechanical explanations of the onset of transverse orientation so also we cannot accept mechanical explanations of its maintenance. Thus the ingenious idea so often figured by Frey-Wyssling (33(*b*)), in which the cell wall is idealized into a network of fibrils with diamond-shaped interstices (Fig. 67(*a*)) and growth is made possible by a "loosening" of the points of crossing (Fig. 67(*b*)) which are otherwise fixed, is indeed hardly acceptable *as it stands.* If the length AC has increased to $A'C'$, then surely the distance $A'N'$ should be proportionately greater than AN—which is impossible without a change in the orientation of AB.

It seems much more feasible to seek for an explanation at a more fundamental molecular level. Up to about fourteen years ago, when the discovery of the "crossed fibrillar "structure was made, orientation was considered to obtain as the result of a pseudo-crystallization of glucose

or some oligo-saccharide or pre-existing oriented chains. Even at the time, of course, this was not a "first cause", but the periodic switches in orientation now known to occur in so many cell walls show at least that some other mechanism is from time to time invoked. This mechanism can hardly lie anywhere except in the protoplasm.

So much was clear in 1937, and since that time it has become progressively more probable that orientation in the cellulose envelope implies orientation in the protein chains, both within a growing wall

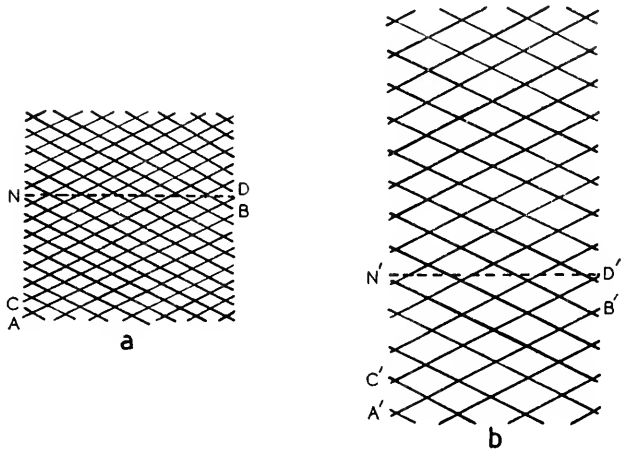


FIG. 67. Diagrammatic representation of the run of the fibrils in a primary wall (*a*) before, (*b*) after a period of growth. The fibrils are supposed to maintain their orientation constant by slip at the points of crossing (after Frey-Wyssling).

and in the cytoplasmic surface during secondary wall deposition. The molecular configuration for normal and for supercontracted wool keratin suggested by Astbury(65), made it possible even to hazard a guess as to how protein chains might readily be responsible both for the almost transverse orientation in growing walls and the almost longitudinal orientation in adult walls(66). During periods when the orientation of the cellulose chains in a wall layer remains constant, it is still possible that the "crystallization" forces of the cellulose matrix assume priority; but once the orientation changes then it is certain that the protoplasmic mechanism has taken control. It is much too early in the history of this most important aspect of the study to make any definite pronouncement, but there is already in the literature a number of interesting pointers. Much of this subsidiary evidence refers to the animal rather than to the plant cell; we should not thereby be

discouraged, for it seems unlikely in the extreme that the structural features of plant protoplasm can be essentially different from that in animals. By the time that the *Cladophora* study had been begun it was already known that there were isolated examples of what looked like protein orientation in the cytoplasm of the algae. Recently, however, this possibility has been enormously strengthened through the investigation by Picken(67), using optical and X-ray methods much as described in this book, of the growth of Lepidoptera scales. In the adult scales Picken has shown what Rudall(68) had already found for insect cuticles, that protein and chitin chains in the chitin-protein complex are similarly oriented to some morphological axis. Even more important than this, however, Picken has obtained birefringence data which suggest that, at an early period of growth when the structures concerned are mainly protein, the protein chains are nevertheless oriented parallel to the direction of growth. This lends further support to the statements by Monné(69) and by Schmidt(70) that the fine structure of the cytoplasm is a function of the form of the cell. The growing protein-chitin complex resembles in some respects the cellulose-protein complex which we have found to be present in the growing plant cell wall. The earlier development of the protein suggests clearly that this, as it were, forms the template upon which the subsequent molecular chains, of chitin in the one case and cellulose in the other, are oriented. This is, of course, as yet pure speculation but, if it turns out to be true, then the evidence before us at the moment would suggest that the spatial relationships between cellulose and protein might be different from that between chitin and protein. For whereas chitin chains like the proteins seem to be oriented parallel to the direction of growth, cellulose chains are often laid down perpendicular to this direction. The different association of chitin and cellulose might well arise through the $—NHCOCH_3$ groups of the former.

If our assessment of the present position is correct, then it seems rather likely that the proteins of the cytoplasm form an organized system which is responsible for the orientation, as well as the construction, of the cellulose chains. This makes it further possible to suggest that the proteins concerned, whether in the growing cell or in the later development of a secondary wall, are of the nature of carbohydrases. If this is so, then it becomes considerably easier to conceive of the increase in area of the wall during growth without reorientation; for the whole structure is then very labile and we could imagine the breaking of bonds and the insertion of new material in such a way that

no strain is thrown upon existing cellulose fibrils. The extreme apical growth in *Phycomyces* which we have discussed above, and the similar cases reported in the literature, suggest that in many cells this process is strictly localized.

Some evidence for the localization of cellulose synthesis during wall thickening has already been presented (42(*b*) and (*d*)) but it is not, of course, by any means certain that such considerations can be carried over to the growing wall.

There still remains to be considered the apparent independence, in many cases, of the form of the cell from wall structure, and perhaps species like *Phycomyces* give us a clue. If the making and breaking of bonds occurs solely at one end of a cell then the cell will of necessity enlarge into a cylinder. Whether or not the cylindrical form is adopted would then depend on whether or not the hydrostatic pressure within the cell is sufficient to cause the mutual displacement of the broken or separated threads necessary for the insertion of new material. This would make the deposition of cellulose, and its incorporation in the wall as part of an organized structure, two rather independent processes. It is therefore with profound interest that we note the observation made recently by Gorter(71) that treatment of root hairs with tri-iodobenzoic acid inhibits elongation but does not prevent cellulose deposition at the tip of the hair.

It is not, however, possible to refer all cases to apical growth. To take the two cell types studied mainly in this chapter, apical growth *sensu strictu* has never been suggested for the parenchyma of oat coleoptiles* though a great deal of evidence has from time to time been brought forward to show that in similar cells within the root the various parts of the walls of any one cell do not grow simultaneously; and in cambial cells, though apical growth has been adduced in order to explain features of growth into which we cannot go here, there is no very clear evidence on this point. Growth of this particular kind is not, however, essential. It seems very unlikely indeed that all parts of the wall of a cell are undergoing the same processes simultaneously—for one thing, the cell would probably burst if that were so. It seems much more likely that the breaking of bonds, the intercalation of new material, and the making of bonds occur, in any one instant of time, only in isolated patches of the wall. In the next instant, the processes will be taken up by other patches, and so on. The form of a growing cell will then depend on the spatial distribution of these patches, on the way in which the new material is inserted (and therefore often, though not necessarily

* This suggestion has, however, now been made by Frey-Wyssling (73).

invariably, on the orientation of existing material) and, within a tissue, on surrounding conditions which may impose limits on expansion in some direction.

Such conceptions are naturally of value only insofar as they will stand up to rigid comparison with data obtained by rigidly controlled experimental treatment of growing cells. An attempt to make such a test is well outside the scope of this book but we may perhaps refer very briefly to perhaps the most interesting growth study which has yet appeared. Working with root segments in which growth occurred by vacuolation only, and therefore free from internal control either by neighbouring dividing cells or by the exigencies of a shoot system, Brown and Sutcliffe(72) have established the following facts. The segments will almost double their length when placed in water alone, in a period of some twelve hours. In sugar solutions, however, the total elongation obtainable is much greater, reaching a length about four times the original in a period of about 48 hours. This greater length is achieved, not by an increase in the *rate* of growth but in the *time period* during which growth proceeds. If potassium ions are added to the sugar solutions, then still greater extensions are achieved, but now the effect is on *rate* of growth. This effect of potassium is traced partly to an effect on sugar uptake but, and with higher significance, to an effect on respiration. Cellular respiration can therefore provisionally be associated with *rates* of growth. In terms of the suggestions made here, this would be understandable as an effect of respiration on the breaking of bonds in the structural components in the wall, and it may be significant that cessation of growth in the water is associated with a depression of the rate of respiration. The influence of sugar would then be expected to be largely on the *period* during which growth occurred, since growth could proceed only so long as new material could be intercalated. This experimental separation of *rates* from *periods* of growth is therefore not out of harmony with our general ideas derived from structural considerations.

Here, however, we are going well beyond the bounds of existing knowledge. We have come a long way from our original considerations of the dead cell walls which formed the starting point of our study. From our approach to the study of the static structure of mature cell walls we have found ourselves led into a dynamic study of protoplasmic activity. These are the lines upon which future research will develop, and if the attitude adopted in these last pages errs perhaps on the side of insecure speculation it is nevertheless through self-indulgence of this kind that advances are made.

It is fitting therefore to close this account, as we began it, with the words of Nehemiah Grew written more than 260 years ago:

> "To conclude, if but little should be effected, yet to design more can do us no harm; For although a Man shall never be able to hit *Stars* by shooting at them; yet he shall come much nearer to them, than another that throws at *Apples*."

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